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J952-43

IDENTIFYING AND CONTROLLING IMMUNOTOXIC SUBSTANCES

BACKGROUND PAPER

CONGRESS OF THE UNITED STATES
OFFICE OF TECHNOLOGY ASSESSMENT

Recommended Citation:

U.S. Congress, Office of Technology Assessment, *Identifying & Controlling Immunotoxic Substances—Background Paper*, OTA-BP-BA-75 (Washington, DC: U.S. Government Printing Office, April 1991).

For sale by the Superintendent of Documents
U.S. Government Printing Office, Washington, DC 20402-9325
(order form can be found in the back of this report)

Foreword

Thousands of new chemical substances enter the market annually. Although the public continues to embrace the benefits of these substances, increasingly wary consumers now inquire about their downside, particularly health risks. While information about what chemicals are in the air or water and in what quantities is usually forthcoming, answers about their human health effects are often vague and unsatisfying.

Much of the American public – scientists and laymen alike – finds this uncertainty troubling. A recent novel described the impacts of an accidental chemical release on a small community. The following exchange captures the frustration of the townspeople trying to understand the consequences of the chemical exposure:

> "Am I going to die?"
> "Not as such," he said.
> "What do you mean?"
> "Not in so many words."
> "How many words does it take?"
> "Let me answer like so. If I was a rat, I wouldn't want to be anywhere within a two hundred mile radius of the airborne event."
> "What if you were a human?"
> "I wouldn't worry about what I can't see or feel."*

Nowadays, after years of research, answers about potential carcinogens come more readily than those conveyed in the novel. But noncancer health risks, such as potential, adverse effects of chemicals on the nervous, immune, or respiratory systems, have received less attention and remain more of a mystery. The Senate Committee on Environment and Public Works and its Subcommittee on Toxic Substances, Environmental Oversight, Research and Development asked OTA to examine noncancer health risks in the environment, including the availability of testing technologies, future research needs, and the adequacy of the current regulatory scheme. This background paper, which describes Federal efforts to identify and control substances that may harm the immune system, is one response to that request. It builds on previous OTA work on carcinogenic and neurotoxic substances.

OTA acknowledges the generous help of the workshop participants, reviewers, and contributors who gave their time to ensure the accuracy and completeness of this study. OTA, however, remains solely responsible for the contents of this background paper.

John H Gibbons

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Director

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*Don DeLillo, *White Noise* (New York, NY: Penguin Books, 1986), pp. 140-141.

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NOTE: OTA appreciates and is grateful for the valuable assistance and thoughtful critiques provided by the workshop participants. The participants do not, however, necessarily approve, disapprove, or endorse this background paper. OTA assumes full responsibility for the background paper and the accuracy of its contents.

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Chapter 1

Introduction and Summary

Chapter 1

Introduction and Summary

INTRODUCTION

The potential for substances used in industry, transportation, and households to be simultaneously beneficial and toxic to human life creates a legislative and regulatory dilemma. The challenge of balancing a strong economy, one that delivers products that people need and desire, with the health and safety of the populace sometimes seems to be a tremendous burden.

Technological advances add to the weight of that burden. Thousands of new, potentially toxic substances enter the market annually. Advanced instruments help scientists measure the presence of new and existing substances in minute quantities. Substances formerly unknown or undetected suddenly become worrisome as technology provides the means to predict human risks from these substances.

Governmental concern that a substance might create an adverse health effect historically concentrated on carcinogenicity. Most Federal legislative and regulatory efforts to prevent or minimize human exposure to toxic substances have focused on identifying and controlling carcinogens. Physicians and researchers now recognize the noncancer, toxic effects of many substances. Some of these effects, for instance teratogenicity, have become the subject of specific legislative concern. Federal regulatory attention to other types of toxic injury, e.g., to the nervous system, the immune system, the respiratory system, depends on the more general mandate to protect human health. Some observers fear that historical emphasis on carcinogenicity, combined with limited agency resources, has led to neglect of problems such as neurotoxicity, immunotoxicity, or pulmonary toxicity—problems that may be as widespread and severe as carcinogenicity.

The Senate Committee on Environment and Public Works, and its Subcommittee on Toxic Substances, Environmental Oversight, Research and Development, asked for assistance from the Office of Technology Assessment (OTA) in evaluating technologies to identify and control noncancer health risks in the environment. The committee's interests include advances in toxicology, research and testing programs in the Federal agencies, and the consequences of exposure to toxic substances.

This background paper, which describes the state-of-the-art of identifying substances that can harm the immune system, represents one response to the committee's request. Chapter 2 provides basic information about the principal components of the immune system and the general consequences that stem from perturbations to it. Chapter 3 describes methods for evaluating chemical immunotoxicity and reports on some known or suspected immunotoxicants. Chapter 4 summarizes Federal research and regulatory activities related to immunotoxicity. Appendix A provides a very brief synopsis of income replacement programs available to persons disabled by toxic exposures.

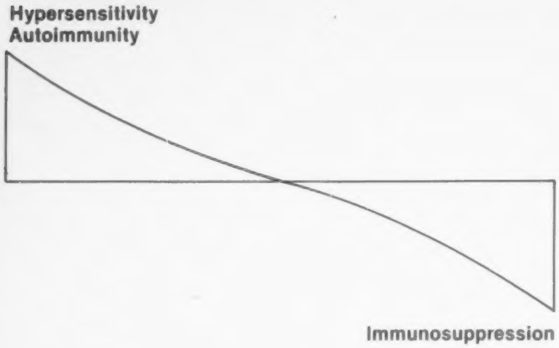
TERMINOLOGY

This background paper examines a field of study rife with jargon. Not all specialists in the field of immunotoxicology—which combines the expertise of immunologists and toxicologists—use terminology in precisely the same manner. The following definitions explain how OTA applies the terms-of-art essential to this study.

Immunotoxicity, or an immunotoxic effect, is defined as an adverse or inappropriate change in the structure or function of the immune system after exposure to a foreign substance. Foreign substances capable of adversely affecting the immune system may be of synthetic or natural origin. This study focuses on chemicals, including industrial, transportation, agricultural, and household chemicals, drugs, and food additives.

Immunotoxicity manifests at both ends of a spectrum—as an enhanced but inappropriate immune response, and as a failure to mount an appropriate immune response. (See figure 1-1.) This background paper examines three types of adverse effects: immunosuppression, hypersensitivity, and autoimmunity. *Immunosuppression* is a generalized decrease in immune responsiveness that may result in increased incidence of

Figure 1-1—Spectrum of Adverse Immune System Effects



SOURCE: Office of Technology Assessment, 1991.

infection or tumors. Immunologic *hypersensitivity* is allergy—an overreaction of the immune system to a substance. Hypersensitivity is a normal immune response to a foreign substance, but one with deleterious consequences. *Autoimmunity* results from a breakdown in the ability of the immune system to distinguish "self" from "nonself." An autoimmune reaction involves the body mounting an immune response against some of its own components.

Substances that provoke an immune response are called *antigens.* Antigens trigger beneficial responses, e.g., destruction of an infectious agent, and adverse responses, e.g., hypersensitivity. Other substances can damage the immune system by destroying or inactivating specific elements within it, thereby suppressing immune response. Substances that elicit adverse immune responses or damage the immune system are called *immunotoxic substances,* or *immunotoxicants.*

THE IMMUNE-SYSTEM

The immune system is a complex, cooperative effort among several types of cells, cell products, tissues, and organs in the body. The simplified description that follows should help the reader comprehend state-of-the-art immunotoxicology, but by no means represents an exhaustive examination of this complicated organ system.

The immune system involves several cell types. Most of them belong to the large group of cells commonly lumped together as white cells. The white cells of principle interest to immunotoxicologists are macrophages and lymphocytes. Macrophages ingest cellular debris and infecting organisms, and they process and present antigens to the lymphocytes. Lymphocytes include B cells, T cells, and natural killer (NK) cells. B cells secrete antibodies (immunoglobulins), substances that inactivate antigens in the body. Each B cell makes antibody specific to a given antigen; the body can make antibodies to thousands of antigens.

T cells regulate the immune system. T cells must interact with B cells for antibody production. Some T cells can directly kill cells presenting antigens. T cells also secrete cytokines, protein molecules that transmit signals to modulate (augment or suppress) immune response between cells of the immune system. (Macrophages also produce cytokines.) NK cells attack and destroy certain other cells. Most types of cells are resistant to NK cell activity, but tumor cells and virus-infected cells appear to be susceptible.

The organs of the immune system—the lymphoid organs—include the bone marrow, the thymus, the lymph nodes, and the spleen, as well as the tonsils, the appendix, and clumps of tissue in the small intestine and the respiratory tract. The lymphoid organs are compartmentalized collections of lymphocytes, macrophages, and other immune system cells. They produce, store, and distribute the immune system cells. The lymphatic vessel network also circulates lymphocytes.

Foreign substances provoke two basic types of immune response: nonspecific immunity and acquired immunity. Nonspecific immunity, which involves functions of the macrophages and NK cells, represents the body's first line of defense. The nonspecific immune response occurs without prior exposure to antigens. Acquired immunity develops after the initial exposure to a foreign substance and can be subdivided into humoral immunity and cell-mediated immunity. Humoral immunity involves antibody production, which generally requires macrophages (to process and present the antigen), T cells (to stimulate B cell production), and B cells (to produce antibodies). The other basic type of acquired immunity, cell-mediated immunity, also begins with the macrophage but then relies on the T cell functions of cytotoxicity and cytokine secretion. Although scientists distinguish between humoral and cell-mediated immunity, most antigens can provoke both types of immune response.

IDENTIFYING IMMUNOTOXIC SUBSTANCES

The AIDS epidemic has heightened public interest in the immune system and its potential for injury. A virus, not a chemical, causes AIDS, but the disease illustrates the devastating effects of immunosuppression and suggests to people the importance of protecting the immune system. In response to increased public awareness, researchers have devoted greater effort in recent years to developing predictive tests to measure the potential immunotoxicity of chemicals before they enter commerce.

As a result of these efforts, a number of tests now exist to assess the effects of foreign substances on the components and processes of the immune system. Researchers testing a chemical's immunosuppressive potential often start with pathology, an examination of tissues and organs of the immune system for evidence of disease. The organs of experimental animals can be weighed or cells from organs or in peripheral blood can be counted to get preliminary indications of how a substance may affect the immune system. Other assays of immunosuppression assess whether exposure to a chemical affects the ability to mount a particular type of immune response. Measures of humoral, cell-mediated, and nonspecific immunity exist and can be applied in the laboratory. These tests usually involve experimental animals but occasionally direct assessment of humans.

The most comprehensive tests of immunosuppression available to researchers today measure a subject's ability to fight infections or tumors after exposure to a suspected immunotoxicant. Scientists expose experimental animals to a toxic substance and subsequently expose (challenge) the animals to an infectious agent or a tumor. If animals exposed to the suspected toxicant show a significant increase in the incidence of disease or death following challenge compared to unexposed animals, the substance is suspected to be immunotoxic.

Researchers and manufacturers also use hypersensitivity assays. Whether a substance can induce hypersensitivity can be assessed using skin and respiratory tests. Antibodies can also be measured in the blood of animals or humans who have had an allergic reaction to an antigen. (Table 1-1 lists common immunotoxicological tests.)

Immunotoxicological testing presents an investigator with significant challenges in interpretation. Test results can differ depending on the subject's age, species, sex, or recent illnesses. Environmental factors, such as diet or other chemical exposures, can also affect immune system performance. Choosing the appropriate test dose and the means and duration of exposure can prove difficult when the point of the exercise is to extrapolate from the test to the predicted consequences of actual human exposure. (See box 1-A.) These considerations present themselves in all types of toxicology, but the complexity of the immune system compounds these difficulties for immunotoxicologists. Immunotoxicology's fledgling status as a field of scientific inquiry and the fact that few toxicologists have training in immunology further complicate study of immunotoxicity.

The immune system is thought to have a reserve capacity, although the size of that reserve is as yet undetermined. Thus tests that measure impairment of one immune system component may not, in fact, indicate overall immunotoxicity, since other immune components or processes may compensate for the impairment. Frequently a decrease in a particular immune function is discerned but no clinical symptoms – certain infections or cancers, for instance – appear during the test period. There is still much to be learned about the long-term consequences of weakening an individual component of the immune system.

Relatively few data exist on immunotoxicity in humans. Studies of radiation victims and patients receiving immunosuppressive drugs provide evidence of the consequences of long-term immunosuppression. A few epidemiologic studies of toxic exposures that appeared to result in immunosuppression have been attempted,

Table 1-1—Common Immunotoxicological Tests

Category	Test
Pathology	Hematology
	Organ weights
	Histology
	Cellularity
Humoral immunity	Antibody plaque forming cell (PFC) response
	B cell mitogen response
	Immunoglobulin levels in serum
	Quantitation of splenic and/or peripheral blood B cells
Cell-mediated immunity	T cell mitogen response
	Cytotoxic T lymphocyte (CTL) cytolysis
	Delayed hypersensitivity response
	Mixed leukocyte response (MLR)
	Quantitation of splenic and/or peripheral blood T cells
Nonspecific immunity	Natural killer cell activity
	Macrophage counts
	Neutrophil counts
Host-resistance	Syngeneic tumor cells:
	• PYB6 sarcoma (tumor incidence)
	• B16F10 melanoma (lung burden)
	Bacterial models:
	• *Listeria monocytogenes* (mortality)
	• *Streptococcus* species (mortality)
	Viral models:
	• Influenza (mortality)
	• Mouse cytomegalovirus (mortality)
	Parasite models:
	• *Plasmodium yoelii* (parasitemia)
Hypersensitivity	Draize test
	Open epicutaneous test
	Buehler test
	Freund's complete adjuvant test
	Optimization test
	Split adjuvant test
	Guinea pig maximization test
	Mouse ear swelling test
	Respiratory rate measurement
	Serum IgE measurement
	Local lymphnode proliferation assay

NOTE: Immunotoxicologists seldom perform each test or type of test for a particular substance. They often divide the tests into *tiers*, using tests that indicate immune system damage at a fairly gross level to screen for potential immunotoxicants, and applying more sensitive and specific tests only to those substances that indicate possible toxicity in the primary screen. These tests are described in ch. 3.

SOURCE: Office of Technology Assessment, 1991.

but with inconclusive results. On the other hand, workers and consumers have provided conclusive evidence that several chemicals can induce hypersensitivity. At least one epidemiologic study has examined humans for signs of autoimmunity resulting from chemical exposure. However, the overall lack of data makes it problematic to extrapolate from the results of tests of substances in experimental animals to the likely effects of human exposure.

EXAMPLES OF IMMUNOTOXIC SUBSTANCES

Scientists have identified several substances that suppress the immune system, induce hypersensitivity, or cause autoimmunity in laboratory animals. This is true despite the fact that testing for immunotoxicity has been done on very few of the chemical substances, regulated or unregulated, in commerce.

Substances That Suppress Immune Response

Substances that can suppress immune responses fall into several general categories, including: therapeutics, solvents, pesticides, halogenated aromatic hydrocarbons, polycyclic aromatic hydrocarbons, airborne pollutants, and metals. Testing has not been conducted on all individual substances in any particular category, and not all substances tested within each of these categories have been shown to be immunotoxic. Not all categories of chemicals have been scrutinized.

Therapeutic drugs designed to prevent transplant rejection or treat cancer or autoimmune disorders are the most thoroughly studied immunosuppressive chemicals. The most frequently used immunosuppressive drugs fall into four basic categories—alkylating agents (e.g., cyclophosphamide), glucocorticosteroids (e.g., prednisolone), antimetabolites (e.g., azathioprine), and natural products (e.g., cyclosporin A).

Benzene, a solvent commonly used in many industrial processes, has been linked to immune dysfunction. Workers chronically exposed to high levels of benzene (>100 ppm in the air) experienced increased rates of agranulocytosis and myelogenous leukemia, accompanied by an increased risk of infection. The immunotoxicity of benzene at levels closer to the current regulatory level (1 to 5 ppm in the air) has not been demonstrated.

Animal studies of pesticides show evidence of immunosuppressive potential, but little analysis of the effects of human exposure has been done. Among the commonly found halogenated aromatic hydrocarbons (HAHs), polybrominated biphenyls (PBB), polychlorinated

Box 1-A—General Principles of Toxicology

To evaluate the toxic nature of a substance, including its immunotoxicity, scientists have developed several general criteria for consideration, including:

Nature of the Toxic Substance—Toxicologists try to determine the characteristics that render a chemical toxic. Individual molecules may be nontoxic in their native states but become toxic after being metabolized.

Dose and Length of Exposure—These parameters, together with rates of metabolism and excretion, determine what quantity of a substance is actually affecting the body. A given substance may be toxic in high doses but nontoxic under conditions of chronic low-dose exposure.

Route of Exposure—The pathway by which a toxicant enters the body (e.g., skin, eye, lungs, or gastrointestinal tract) affects its toxicity. The amount of absorption, ability of the toxicant to combine with native molecules at the entry point (e.g., heavy metals with skin collagen), vulnerability of sensitive areas (e.g., lining of the lung), and condition of the organ at time of contact (e.g., pH and content of the stomach) all play a role in subsequent toxicity.

Species Affected—Toxicants exhibit different levels and effects of toxicity depending on the species on which it is tested.

Age—Susceptibility to a toxicant varies with age—the young and the old generally being the most susceptible.

State of Health—The health status of an individual, including the presence of disease, can greatly affect toxicity response. For example, substances that are detoxified by individuals with normal liver functions may cause adverse effects in people with liver diseases.

Individual Susceptibility—Host factors such as genetic predisposition affect the response of an individual to a toxicant.

Presence of Other Agents—Toxicology often involves evaluating one substance in isolation, yet the body is seldom exposed to agents in this manner. Knowledge about toxic effects of multiple substances is not well-developed because of the practical limitations of testing the infinite number of combinations.

Adaptation/Tolerance—Biological adaptation to a toxicant often occurs when chronically low doses are presented. Adaptation/tolerance must be factored into evaluating the range of individual responsiveness to a toxicant.

SOURCE: Office of Technology Assessment, 1991, based on M.A. Ottoboni, *The Dose Makes the Poison* (Berkeley, CA: Vincente Books, 1984).

biphenyls (PCBs), and dioxin have shown evidence of immune suppression in animal studies. However, the few human studies on these substances have either been inconclusive or have contradicted the animal evidence.

Carcinogenic polycyclic aromatic hydrocarbons (PAHs), the products of fossil fuel combustion, create clear signs of immunosuppression in animal studies. Humans are exposed to PAHs in the workplace and as airborne pollutants, but few data on their effects on humans exist. Other airborne pollutants with immunosuppressive potential include ozone, nitrogen dioxide, cigarette smoke, and trace metals. Metals shown to suppress immune function in experimental animals include lead, cadmium, mercury, and organotins.

Substances That Induce Hypersensitivity

Contact sensitivity and other immune mediated skin disorders often arise from exposure to environmental agents. A variety of substances, including drugs, cosmetics, and certain metals can give rise to allergic contact dermatitis, which manifests itself with a red rash, swelling, itching, and sometimes blisters. Numerous inhalants cause immune-mediated respiratory disorders, including some types of asthma, hypersensitivity pneumonitis, allergic rhinitis, bronchopulmonary aspergillosis, silicosis, asbestosis, coalworkers' pneumoconiosis, and possibly byssinosis.

Among the known human allergens are certain prescription drugs, including penicillin and methyldopa. Over-the-counter drugs, cosmetics, and the metals found in costume jewelry can also cause allergic reactions. Plastics and resins, particularly the isocyanates, cause both asthma and contact dermatitis. Pesticides, too, have provoked hypersensitivity under certain conditions.

Substances That Induce Autoimmunity

Several drugs and heavy metals have been implicated in autoimmune processes. Genetic susceptibility also plays an important role in autoimmunity. Because of this strong genetic component and a generally poorer understanding of autoimmunity compared to other immune responses, deciphering the exact role of toxic chemicals in the induction of autoimmunity is difficult.

FEDERAL ATTENTION TO IMMUNOTOXIC SUBSTANCES

The Federal Government is actively involved in advancing the state-of-the-art of immunotoxicology. The Environmental Protection Agency (EPA), Food and Drug Administration (FDA), Centers for Disease Control, and National Institutes of Health have immunotoxicological research programs. Each of these agencies also contributes to the work of the National Toxicology Program (NTP), whose immunotoxicological research program was the first coordinated Federal effort to evaluate toxic substances for adverse effects on the immune system.

Current Federal research in immunotoxicology is directed toward developing and validating tests for evaluating substances for immunotoxic potential. NTP has published a panel of tests for immunosuppressive potential that has been validated in the mouse, and it continues to work on validating immunotoxicity tests in other species and on improving its current panel of tests. NTP is also applying the battery of tests for immunosuppressive potential and standard hypersensitivity assays to various substances. EPA is working independently and with NTP on developing and validating immunotoxicity tests using the rat and the mouse, and has undertaken human inhalation studies on substances regulated under the Clean Air Act. EPA has also published immunotoxicity testing guidelines for certain pesticides. FDA considers the immune system an important target of toxicological assessment and is involved in developing and applying tests to measure the potential immunotoxicity of foods and drugs. (See table 1-2.)

Few substances have been regulated by the Federal Government on the basis of immunotoxicity. The Occupational Safety and Health Administration (OSHA) has issued regulations for certain substances because they can provoke hypersensitivity. FDA has restricted the use of sulfites in foods because they can induce asthmatic attacks in sensitive individuals. These agencies and EPA regulate additional substances that have shown evidence of immunotoxicity, but other health effects serve as the basis for those regulations.

Several Federal activities are designed to enhance public awareness of the hazards of toxic substances, including immunotoxicants. OSHA's hazard communication standard requires that workers be provided with information about known health hazards in their jobs. However, since so little information is available regarding immunotoxic effects, and since the standard cannot be used to compel testing, the standard does little at present to protect workers from immunotoxic hazards. Community right-to-know legislation requires EPA to collect information about substances that pose potential toxic hazards to local communities and make that information available to the public. As with the OSHA stan-

Table 1-2—Federal Agencies Engaged in Immunotoxicological Research or Regulation

- Consumer Product Safety Commission
- Department of Agriculture
- Department of Defense
- Department of Health and Human Services
  - Agency for Toxic Substances and Disease Registry
  - Alcohol, Drug Abuse, and Mental Health Administration
  - Centers for Disease Control
  - Food and Drug Administration
  - National Institutes of Health
- Department of Labor
  - Mine Safety and Health Administration
  - Occupational Safety and Health Administration
- Environmental Protection Agency

SOURCE: Office of Technology Assessment, 1991.

dard, however, this program does not permit EPA to require that health effects information be developed. The Federal Government also supports information dissemination activities related to toxic substances undertaken by the National Library of Medicine and the Agency for Toxic Substances and Disease Registry.

RELATED ISSUES

As a science develops, experts continually disagree on whether currently available evidence supports a particular conclusion. In the case of immunotoxicology, a growing number of medical practitioners are drawing conclusions regarding the immunotoxicity of chemicals in the environment that other practitioners and researchers believe to be unjustified on the basis of current knowledge. This disagreement has had at least two interesting effects on governance in the past few years.

One effect is the increasing public demand for governmental attention to a problem frequently identified as multiple chemical sensitivity (MCS) but also called environmental illness and several other names. MCS remains a poorly defined problem—experts disagree on what causes the problem and whether it is physical or mental—but sufferers generally exhibit symptoms that they attribute to immune system dysfunction when exposed to very low levels of common industrial, environmental, and household chemicals. Two States, Maryland and New Jersey, commissioned studies of MCS as a public health problem but have not proceeded with screening or regulatory programs. California has considered (but not passed) legislation that would encourage research and education regarding environmental illness and MCS. Within the Federal Government, EPA is funding a study by the National Academy of Sciences that will assess the type of research needed to determine whether MCS exists and its possible causes. Constituents at the State and Federal level are appearing before their elected representatives to demand attention to this problem, which some medical practitioners attribute to immunotoxicity. This background paper does not attempt to weigh the merits of arguments supporting or denying the existence of MCS or of claims that MCS is an immune system dysfunction. The analysis examines state-of-the-art methods for identifying substances that may damage the immune system but does not evaluate diseases of or involving the immune system.

A second effect is the introduction of immunotoxicological test results to support claims of disability. Individuals with diseases they believe to be linked to immune system impairment are making claims against Social Security, workers' compensation programs, and in tort suits for injuries caused by chemicals frequently used in commerce. Here, too, experts disagree on whether available evidence supports these claims. However, most of the experts contacted by OTA believe that data currently available from immunotoxicological testing are limited in the support they provide to plaintiffs' claims of immune system injury from toxic substances. Appendix A provides a brief description of the compensation programs and a synopsis of a few claims based on immune system injury from toxic chemicals.

SUMMARY

Immunotoxicology has advanced rapidly as a distinct field of study over the last several years. Scientists now recognize the immune system as an important target organ for toxicity. Tests to determine whether a substance can suppress the immune system or induce hypersensitivity or autoimmunity have been developed and applied by manufacturers and by regulatory agencies. Yet the growing database of immunotoxicological information remains insufficient for most regulatory purposes.

Immunotoxicology is complicated by several factors: the complex nature of the immune system, limited (historically) attention to immunotoxicity research, and lack of human exposure data stand out among them. Regulators at the FDA have the opportunity to observe the effects of drugs on humans in clinical trials before they are marketed. However, when FDA, EPA, OSHA, or the other regulatory agencies try to decide whether a food additive or pesticide, for instance, poses an undue risk to human health, they face the problem of trying to extrapolate from animal test results to the likely consequences of human exposure to immunotoxic substances. Very few useful human data are available to support these attempts.

The science of immunotoxicology is sufficiently advanced for regulators to begin to consider use of its results in the decisionmaking process, but much research remains to be done. Current law permits Federal agencies to require immunotoxicological testing—either by the government or by industry—on substances suspected of posing a potential threat to the human immune system. The agencies are devoting resources to developing and applying immunotoxicological tests in areas where the potential for human exposure is thought to be significant.

Chapter 2

The Immune System and Immunotoxicity

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INTRODUCTION

Each day, the human body must defend itself against a battery of foreign substances (e.g., bacteria and viruses), as well as against elements from within (e.g., cancer cells). This protective capacity arises, for the most part, from our immune system, which can generally distinguish what is "self" from what is "nonself," or foreign. The stark statistics of acquired immune deficiency syndrome (AIDS), for example, demonstrate the importance to human health of an appropriately functioning immune system.

The immune system is a complex, highly regulated, cooperative effort among several types of cells, cell products, tissues, and organs. It is not an isolated system, but operates in concert with other systems within the body (e.g., endocrine, central nervous, and cardiovascular). Any particular stimulus to the immune system results in a complex series of events, and any single response can have a number of consequences. Similarly, perturbations that primarily affect other body systems can cascade, resulting in an effect on the immune system.

This chapter briefly describes the principal components of the immune system and the general consequences that stem from perturbations to it. This chapter also briefly explores one of the practical considerations of immunotoxic substances and humans: Who is at risk?

Only the most basic and broad concepts of immunology are presented in this chapter. Current knowledge about the immune system is presented in several sources (4,19,22). Similarly, a detailed description of toxicological effects on the immune system is beyond the scope of this report but is presented elsewhere (7,10,11,18,27,28).

COMPONENTS OF THE IMMUNE SYSTEM

Cells are the basic structural unit of living organisms. They are the smallest components of plants and animals that are capable of carrying on all essential life processes. A single cell is a complex collection of molecules with many different activities, all integrated to form a functional, self-assembling, self-regulating entity, although cells in different organs carry out different functions.

Several types of cells comprise the human immune system, as do units of cells and tissue, i.e., organs. Together these cells, tissues, and organs respond to challenges to the immune system. This section briefly describes the major components of the immune system; a later section examines how these elements coordinate to mount an immune response.

Antigens and Antibodies

Elements that are capable of eliciting an immune response are called antigens. Humans are exposed to antigens primarily through inhalation, direct skin contact, or ingestion. Antigens include, but are not limited to, chemical compounds and micro-organisms. And, while antigens are generally substances "foreign" to – i.e., originating outside – the human body, in some instances, the immune system will perceive a normal, self component of the body as foreign and mount an immune reaction against it. Several types of cells can react with antigens, depending on the exact nature of the particular antigen.

One response to antigen stimulation results in the production of proteins called immunoglobulins, or antibodies. Antibodies have binding sites that are specific for, and complementary to, the structural features of the antigen that stimulated their formation. Antibodies formed by a sheep, for example, in response to injection of human hemoglobin (the antigen) will combine with human hemoglobin and not an unrelated protein such as human growth hormone.

By combining specifically with antigens, antibodies can neutralize substances, such as toxins. Antibodies also facilitate the elimination of bacteria and viruses to which they are bound by recruiting other components of the immune system (e.g., macrophages and complement).

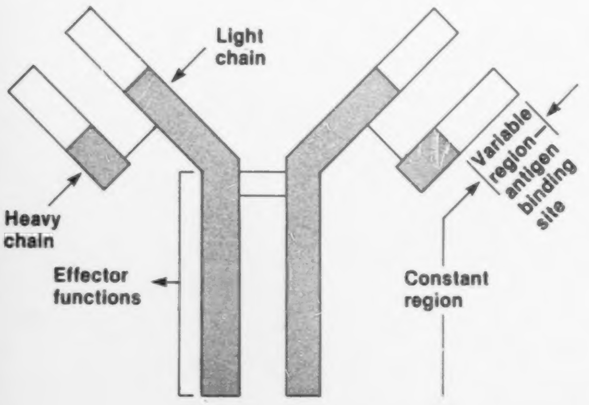
(Complement activation is a rapid, complex process that is activated by antigen-antibody complexes to destroy cells, bacteria, or viruses, or cause inflammation.)

The basic unit comprising antibodies consists of four protein chains – two identical light chains and two identical heavy chains. Heavy chains can vary in type, and the type of heavy chain in a particular antibody determines the subclass – i.e., IgM, IgA, IgD, IgG, or IgE – of antibody. These protein subunits are linked in a fixed and precise orientation to form a "Y"-shaped molecule (figure 2-1). The forked end of the antibody contains two variable regions, the sites of the molecule that recognize and bind with the specific antigen. To accommodate the many antigens that exist, these variable regions differ from molecule to molecule. The other end is nearly identical among all antibodies and is known as the constant, or effector, region. The constant region is not responsible for antibody binding specificity, but has other functions, including promoting efficient attachment of the antigen-antibody complex by certain cells and activation of the complement cascade.

Cells

In recognizing and reacting to substances it perceives as foreign, the immune system, or lymphoid system, involves several different cell types. Most of these cells are commonly referred to as white cells, or leukocytes. Figure 2-2 presents one simple classification scheme for white cells.

Figure 2-1 – Structure of an Antibody Molecule



SOURCE: Office of Technology Assessment, 1991.

An exhaustive description of each type of cell involved in an immune response is beyond the scope of this report. Rather, this section provides short descriptions of only the principal cell types – lymphocytes and macrophages – to serve as background for the following section, which describes the interaction of these cell types, and for chapter 3, which discusses how scientists test for immunotoxicity by measuring perturbations in these cell populations. Table 2-1 lists additional cell types that have immunological importance.

B Cells

A particular subclass of lymphocytes, called B lymphocytes or B cells, recognizes antigens as foreign substances and responds by producing antibodies specific for a given antigen. Antigen-antibody mediated reactions are known as humoral immunity. Once a B cell has been activated by an antigen, it is committed to producing antibodies that bind to the specific antigen. B cells arise from precursors in the bone marrow (as do all other lymphoid and blood cells). These cells, like macrophages (described in following section), play important roles in antigen processing, one sequence of events necessary to trigger antibody production and other immune responses.

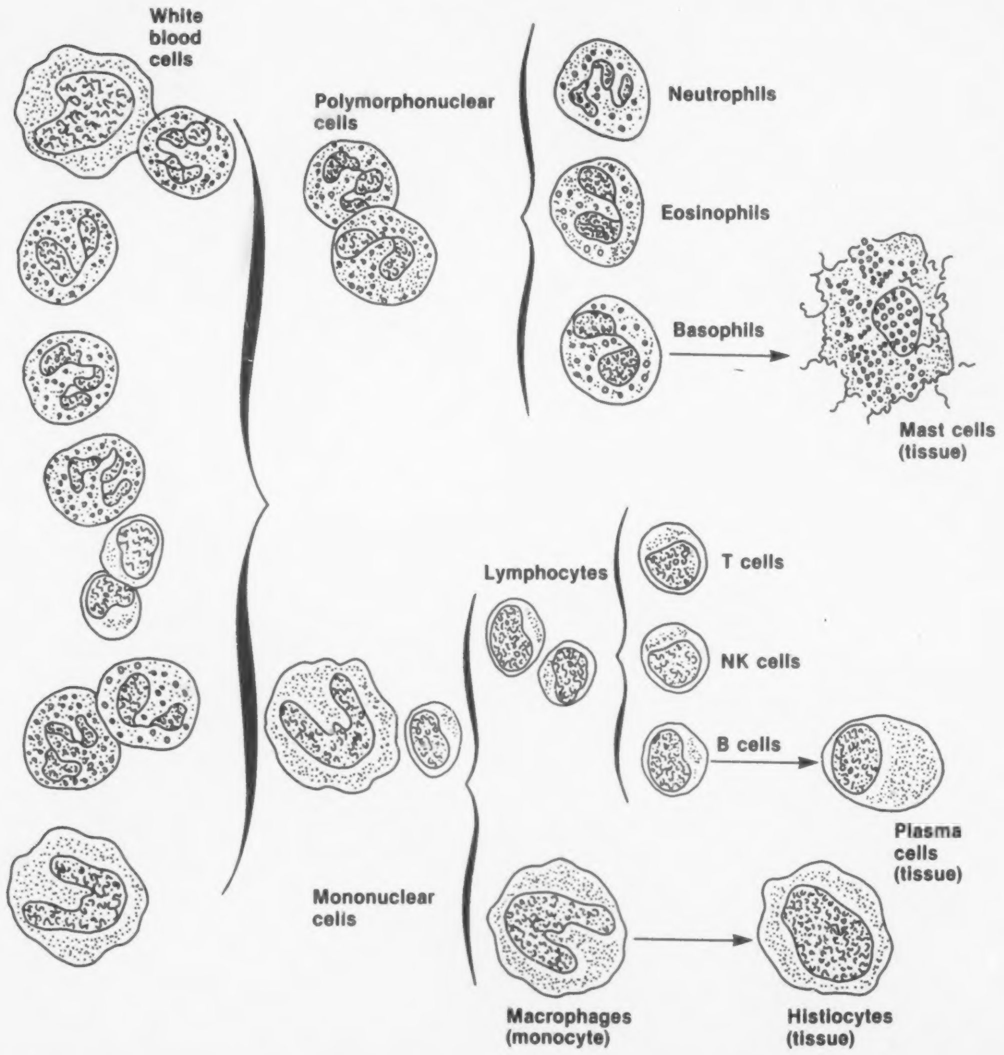
A distinguishing feature of the outer surface of B cells is the presence of surface antibodies. These surface markers, along with surface markers on other cells of the immune system (box 2-A), play critical roles in the activation and

Table 2-1—Major Cells Classes of Immunological Importance

Primary effector cells
B lymphocytes
T lymphocytes
NK cells
Antigen presenting cells
B lymphocytes
Macrophages/monocytes
Follicular dendritic cells
Interdigitating dendritic cells
Langerhans cells
Endothelial cells
Accessory cells
Neutrophils
Basophils and mast cells
Eosinophils
Platelets

SOURCE: Office of Technology Assessment, 1991.

Figure 2-2—White Cells



SOURCE: Office of Technology Assessment, 1991, adapted from S. Sell, *Basic Immunology: Immune Mechanisms in Health and Disease* (New York, NY: Elsevier, 1987).

regulation of the immune system. Surface immunoglobulins define the specificity of the response for that B cell and help trigger the production of antibodies by that cell.

T Cells

A second broad group of lymphocytes are T cells. Like B cells, T cells arise from precursors in the bone marrow, but must circulate to the thymus for maturation. Similarly, they have characteristic cell surface markers and can specifically recognize antigen if it has been processed and presented to them by other cells of the immune system. Based on functional and other criteria, T cells can be divided into several major subpopulations (table 2-2).

T cells can be viewed as the "master regulators" of the immune system (22). In addition to their required interaction with B cells to produce antibody, various T cell types are responsible for cell-mediated immunity, a class

Box 2-A—Cell Surface Receptors of the Immune System

Outer surfaces of all cell types are not smooth, but are imbedded with components called receptors. Hundreds of cell receptor types allow cells to attach to other cells, communicate, bind, or transport drugs from the exterior to a cell's interior, and perform an array of additional functions. Three families of cell surface receptors are "signature" markers for the immune system and regulate the interactions of cells activated during immune responses: the immunoglobulin and immunoglobulin-like family, including antigen receptors on B and T cells; the "integrin" family, which plays an important role in adhesion and migration of immune cells; and the "selectins," which are important for the regulation of certain immune cells at sites of inflammation.

Researchers have begun to unravel how receptors interact with other cells, tissues, and organs of the immune system, and with tissues and organs of other systems. They are also beginning to understand the genetic basis for subtle, genetically based variations in receptors among individuals.

Certain receptors and receptor subclasses are clearly linked to specific immune functions and reactions. This knowledge makes it easy to track these cell types in individuals, to measure how they might differ in the population, and perhaps, to correlate changes or differences with immunoresponsiveness, including susceptibility to disease or a particular toxicant. For example, two immune system receptors on T cells called CD8 and CD4 differentiate cytotoxic/suppressor T cells from helper T cells. Blocking both receptors in laboratory experiments prevents signaling that a "proper" immune response should be mounted, and a 100-fold higher concentration of antigen is necessary to induce a response.

Because the perturbations of many immunotoxicants might take place by alterations of an immune system receptor, or disruption of communication between receptors, understanding immune system cell surface receptors—their structures, functions, and interactions—will likely be important to developing refined immunotoxicity tests. For example, toxicants might exist that block the signaling process. A validated immunotoxicity test to specifically measure signal interruption might be a sensitive and useful tool. Similar tests could be developed based on understanding of integrins and selectins. Such developments, however, are not on the immediate horizon.

SOURCE: Office of Technology Assessment, 1991, based on T.A. Springer, "Adhesion Receptors of the Immune System," *Nature* 346:425-434, 1990.

of immune responses that does not depend on antibody production. T cells can be cytotoxic, i.e., directly kill cells that are expressing a specific antigen. T cells (and macrophages and natural killer (NK) cells) produce a variety of protein molecules, called cytokines, but they do not produce antibodies.

Cytokines serve as messengers that transmit signals between cells to control and modulate an immune response. For example, some cytokines recruit other cells to participate in and augment an immune response. Some stimulate B cells to produce antibodies. Others suppress the immune reaction or ensure that the system focuses on the antigen and does not run rampant in a nonspecific attack that would damage host tissue. Cytokines are produced in extremely small amounts—on the order of parts per billion—and act locally at the site of their release. The interferons are examples of cytokines.

Macrophages

Macrophages (literally "large eaters") are the largest cell type in the immune system and play a central role in the immune system's response to a foreign agent. Macrophages invade sites of inflammation and serve to clear the sites of cellular debris; they are also particularly effective in phagocytizing, or ingesting, infecting organisms.

Macrophages do not produce antibodies. Instead, they are involved in the first step leading to induction of an immune response. A macrophage ingests and nonspecifically processes antigen, and then presents the processed antigen on its cell surface so that it can be recognized by specific antigen reactive cells. Such antigen presentation and processing—which is linked to genetic elements (3,12,17,24,29)—is a critical part of the induction of both humoral and certain cell-mediated

Table 2-2—Major Functions of T Cells

Abbreviation	Function
T_D	T cells responsible for "delayed-type hypersensitivity." After reacting with antigen, T_D cells release cytokines that can kill target cells in vitro (cytotoxins) and activate macrophages, inducing inflammation.
T_H	T helper cells assist in humoral immunity, i.e., antibody production. They are required if a B cell is to produce antibody. One characteristic feature of helper T cells is a surface marker called CD4.
T_K	Often referred to as killer T cells, or cytotoxic lymphocytes, this subclass of T cells, after being specifically sensitized, directly interacts with target cells to kill them in vitro. They are distinguished from NK cells by the requirement for sensitization and cell surface markers.
T_S	T cells that serve to control immune responses by producing factors that can act on both B cells and T_H cells. Suppressor T cells can be dis-tinguished from helper T cells because they exhibit a signature molecule referred to as CD8.

SOURCE: Office of Technology Assessment, 1991.

immune responses. (B cells also process antigens.) Macrophages also produce important cytokines that regulate overall immune responsiveness.

NK Cells

Another lymphocyte subclass is natural killer cells, or NK cells. NK cells derive from the bone marrow, but their exact relationship to B cell and T cell precursors remains to be elucidated. NK cells share some properties of T cells and macrophages, but also share several surface characteristics with B cells.

NK cells do not play a central role in antigen-specific antibody responses, but are critical components of general, nonspecific immune defenses. In vitro, NK cells lyse, i.e., kill, several types of tumor cells and virus-infected cells, and thus are believed to be important in the body's defense against viruses or cancer. Additionally, these cells likely play an important role in immunity to certain bacterial and parasitic infections.

Organs

Several organs are responsible for the development and maintenance of a functioning immune system (figure 2-3), which complicates making assessments of immunotoxicity. Changes in the circulatory or lymphatic systems, for example, can affect any number of organs. Strictly speaking, however, lymphoid organs are the principal organs of the immune system (22). A lymphoid organ is a compartmentalized collection of lymphocytes, macrophages, and other immune cells. It may be part of, or mixed among, cells of another organ. Lymphocytes also circulate freely through the blood and lymphatic vessel network.

Bone marrow, the thymus, and even the fetal liver, in part, are lymphoid organs. In humans (and most other species), bone marrow is the predominant organ from which lymphoid cells originate. Some of the cells that develop in the bone marrow complete their maturation before entering circulation. Others, however, must undergo further differentiation in other organs (e.g., the thymus).

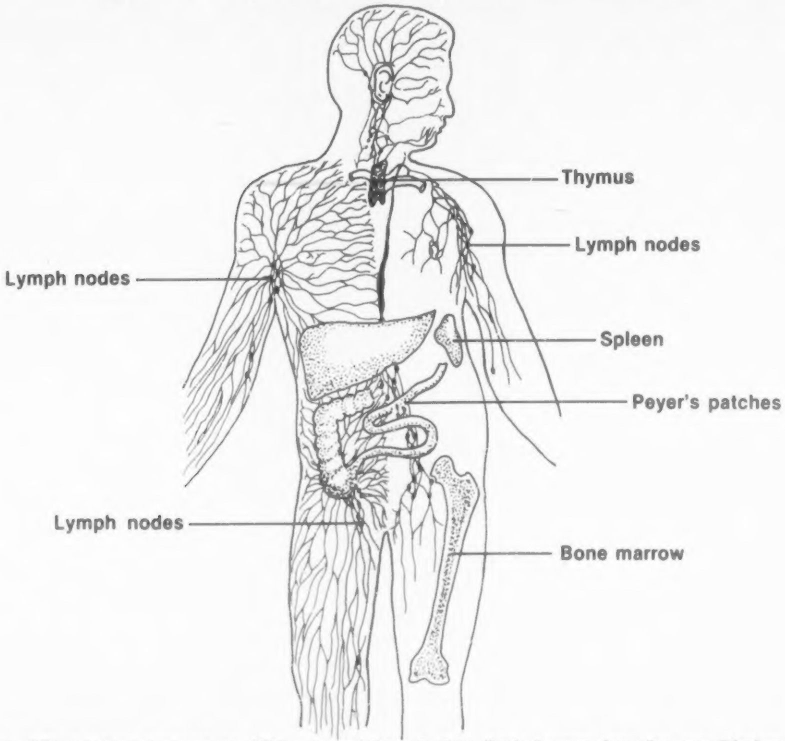
The spleen and lymph nodes are lymphoid organs that clear the blood of infectious organisms and serve as repositories for antibody-producing cells. Tonsils, the appendix, and Peyer's patches (small aggregates of cells in the intestine) are also lymphoid organs. Local collections of tissue within both the gastrointestinal tract and bronchus play a role in certain immune responses—gastrointestinal lymphoid tissue is thought to play an important role in immunity to infectious agents entering the body through the mouth, for example.

IMMUNE RESPONSES

Immune responses can be divided into two categories: nonspecific and acquired. Nonspecific, or innate, immunity involves those cells (e.g, macrophages and natural killer cells) and processes (e.g., complement activation) that do not depend on exposure, followed by reexposure, to antigen. Generally, nonspecific immune responses represent the body's first line of defense and involve a generic, innate reaction to foreign substances. Acquired, or adaptive, immunity is characterized by antigen specific processes.

There are two broad classifications of acquired immunity that are commonly referred to as humoral immunity and cell-mediated immunity. Both require the coordination of multiple cell-types of the immune system. And, while one type of immune response can sometimes dominate after exposure to a particular antigen, for the most part, both play roles when foreign substances are presented. This section briefly describes the interac-

Figure 2-3 – Major Organs and Tissues of the Immune System



SOURCE: Office of Technology Assessment, 1991, adapted from S. Sell, *Basic Immunology: Immune Mechanisms in Health and Disease* (New York, NY: Elsevier, 1987).

tions involved in the two classes of acquired immune responses to provide a background for how perturbations in immune responses can be measured.

As mentioned previously, the immune system functions principally to protect the body. Under some circumstances, however, control mechanisms can be compromised and the immune system turned against the body, causing harm. Conditions that arise when this occurs vary from mild discomfort to fatal disorders. Thus, this section also examines pathological immune responses.

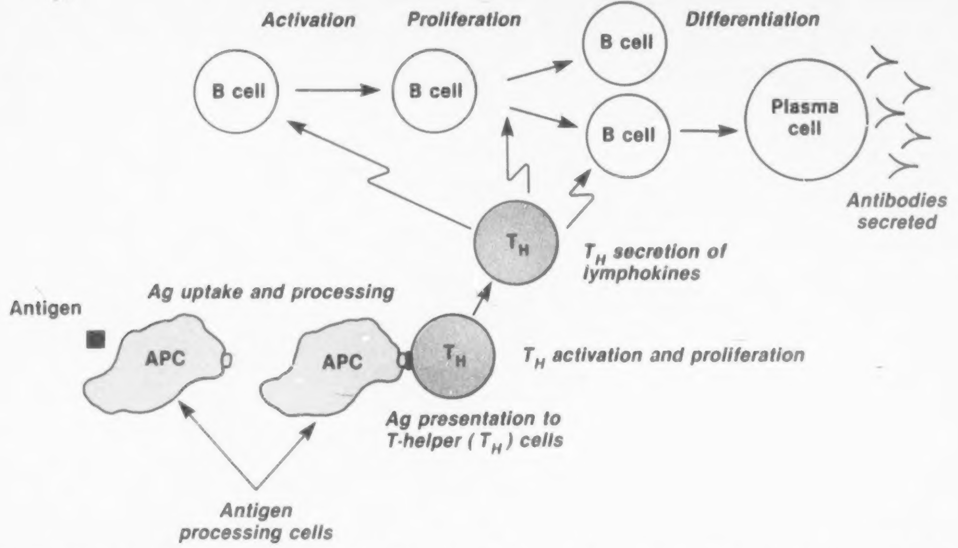
Humoral Immunity

The production of specific antibodies in response to exposure to a foreign substance is called humoral immunity. Humoral immunity can be particularly important in protecting the body against certain biological agents. As illustrated by figure 2-4, three types of immune cells typically interact during optimum immunoglobulin production. A toxic substance that blocks any step can disrupt this response in a measurable fashion.

First, an antigen presenting cell, often a macrophage, must process the foreign substance. Macrophages perform this reaction nonspecifically, and in doing so concentrate the antigen as well as release factors that facilitate subsequent B and T cell cooperation. Second, T helper cells interact with the antigen-presenting cell to produce chemical signals, cytokines (interleukins), that stimulate B cells and additional T cells. Finally, the B cell can mature into an antibody producing cell, also called a plasma cell.

This series of reactions is antigen-specific. Each different antigen elicits a unique antibody and there are a

Figure 2-4 – Generation of Antibody Forming Cells



SOURCE: Office of Technology Assessment, 1991, adapted from K. White, Medical College of Virginia, Richmond, VA, 1990.

large number of antigens against which antibodies can be produced. Additionally, the immune system has a "memory" whereby re-presentation of a familiar antigen results in a slightly different, accelerated immune response. Both B and T cells are involved in immune memory.

In some cases, B cells can be stimulated, by certain antigens called T cell-independent antigens, to produce antibodies without the involvement of T cells. B cells can also be stimulated to proliferate in vitro by mitogens. Mitogen assays are used as a measure of immunotoxicity and are discussed in chapter 3.

One potentially adverse consequence involving antibody production is immediate-type hypersensitivity, a type of allergy response. (Some use the term allergy to refer only to immediate-type hypersensitivity, and not a second reaction, delayed-type hypersensitivity. This background paper uses allergy to denote both responses.) When immediate-type, or anaphylactic, reactions are initiated by certain antigens—e.g., pollens or animal dander—in certain individuals, mast cells (table 2-1, figure 2-2) release pharmacologically active substances (e.g., histamine, serotonin). These substances can induce a range of problems, including inflammatory changes in many tissues that result in hives and wheezing, or even abrupt changes in blood pressure. In some circumstances, such reactions can be life-threatening. The predominant immunoglobulin involved in immediate-type hypersensitivity in humans is called IgE.

Cell-Mediated Immunity

As described previously in table 2-2, several subpopulations of T cells can be recognized physically and functionally. T cells, NK cells, and activated macrophages carry out the functions of the second broad category of acquired immune responsiveness, cell-mediated immunity. Again, the interaction of cells in cell-mediated immunity forms the basis for tests developed to assess potential immunotoxicants. Cell-mediated immune responses require antigen processing to specifically sensitize T cells. Table 2-3 summarizes the general types of cell-mediated immunity. Cell-mediated immunity is particularly important to transplantation rejection, tumor surveillance, and for protection against infectious agents.

Several different types of cell-mediated immune responses exist (8). One potentially adverse consequence of cell-mediated immunity is delayed-type hypersensitivity (DTH). Contact sensitization, such as poison ivy and other skin allergies resulting in inflammation and tissue damage, is a classic example of DTH. Another delayed-type hypersensitivity called cutaneous basophil sensitivity also involves the skin, but the predominant cell type involved differs from that for classic contact sensitivity

Table 2-3—Mechanisms of Cell-mediated Immunity

- Direct killing by sensitized T_K cells; the cells react directly with the target cells to lyse them (cytotoxic T lymphocyte activity).
- Indirect killing by sensitized T_D cells in combination with cytokines.
- Antibody-dependent cell-mediated cytotoxicity (ADCC) involves a type of lymphocyte without characteristic cell surface markers ("null cells") in combination with specific antibody.
- Direct killing by nonsensitized NK cells; the cells react directly with the target cells.
- Phagocytosis, i.e., digestion, of substances by macrophages.
- Release of cytokines.

SOURCE: Office of Technology Assessment, 1991; based on S. Sell, *Basic Immunology: Immune Mechanisms in Health and Disease* (New York, NY: Elsevier, 1987).

and the time lag between exposure and the reaction is shorter (1,8).

Autoimmunity

"Tolerance" is the term applied to the mechanisms that prevent the immune system from responding to its own—i.e., self or auto—antigens. Sometimes, however, tolerance to self antigens is lost, resulting in autoimmune reactions (table 2-4). When such situations occur, humoral immunity, cell-mediated immunity, or both can turn their destructive capabilities against the body, leading to tissue damage and/or inflammation. The presence of antibodies that react against self antigens (autoantibodies) can sometimes be demonstrated in several autoimmune diseases (e.g., systemic lupus erythematosus, rheumatoid arthritis, diabetes). But, the presence of autoantibodies is not inevitably associated with disease and, in fact, autoantibodies normally increase with age.

Autoimmune pathologies can be difficult to assess, but certain features are thought to be indicative of autoimmune phenomena. The induction of autoimmune events in a few discrete populations have been attributed to exposure of the individuals to certain toxicants (5,14,15).

WHAT IS IMMUNOTOXICITY?

As mentioned, immune responses are complex, multifactorial interactions with different consequences depending on the situation. In some instances, the immune response will be depressed or suppressed (table 2-5), which can result in increased susceptibility to disease or tumor formation. Conversely, the immune system sometimes hyperreacts when presented with certain foreign substances, which can result in an allergic response, i.e., immunologic hypersensitivity, wherein the response is out of proportion to and more harmful than the initial threat of the substance. In some of these cases, the response can be the source of tissue damage, so that suppressing certain immune reactions actually reduces tissue injury. Finally, the immune system can become confused and fail to recognize the body's own components as "self"—i.e., the immune system treats a "self" substance as foreign and mounts an autoimmune response, which can result in certain characteristic pathologies.

For the purposes of this study, OTA defines *immunotoxicity* as an adverse or inappropriate change in the structure or function of the immune system after exposure to a foreign substance. Adverse effects can be manifest as immunosuppression, hypersensitivity, or autoimmunity.

In any of these reactions, the cells, tissues, and organs of the immune system can be activated, inhibited, destroyed, or their responses exaggerated. It is the demonstrated involvement of at least one or more of these components that defines whether a consequence results from an actual *immune response*. For example, the term allergy should be used only to denote the involvement of immune responses, but is often mistakenly used to describe other adverse reactions (box 2-B).

An array of biological, physical, and chemical substances can perturb the intricate balance of the immune system, and this complex system provides several target sites for immunotoxicants. **OTA broadly defines *immunotoxicant* as a substance that leads to undesired effects on the immune system. The focus of this report is on immunotoxicants that are chemicals, although important information has been, and continues to be, learned from studies of biological substances.**

Whether a substance causes an adverse effect on the immune system, which could be permanent or reversible, depends on many factors, including the nature of the substance, dose and exposure, route and extent of exposure, the presence of other agents, and an individual's sex, genetic predisposition, age, and state of health.

Table 2-4—Some Human Diseases in Which Autoantibodies Have Been Detected

Disease	Self-antigens (as defined by the autoantibodies involved or detected)
Guillan-Barré syndrome	Myelin and other components of the peripheral nerves
Hashimoto's thyroiditis	Cytoplasmic or microsomal thyroid antigen, thyroglobulin
Multiple sclerosis	Brain or white matter
Myasthenia gravis	Acetylcholine receptor at the neuromuscular synapsis
Progressive systemic sclerosis (scleroderma)	Various antigens in cell nuclei, especially nucleoli
Rheumatoid arthritis	Heart, muscle, joint, subcutaneous nodules, aggregated gamma-globulin
Systemic lupus erythmatosus (SLE)	Various nuclear antigens, especially double-stranded DNA, antigens on leucocytes and erythrocytes, liver, spleen, kidney, muscle

SOURCE: Office of Technology Assessment, 1991; adapted from E. Gleichmann, I. Kimber, and I.F.H. Purchase, "Immunotoxicology: Suppressive and Stimulatory Effects of Drugs and Environmental Chemicals on the Immune System," *Archives of Toxicology* 63:257-273, 1989; and S. Sell, *Basic Immunology: Immune Mechanisms in Health and Disease* (New York, NY: Elsevier, 1987).

Table 2-5—Some Immunodeficiency Disorders

Disorder	Immunological defect
Acquired immune deficiency syndrome (AIDS)	Viral infection of certain T cells
Congenital thymic aplasia (DiGeorge syndrome)	Thymus absent or small; T cells absent or reduced
Severe combined immune deficiency disease (SCID; "Bubble Boy" disease)	Lack of T and B cells
X-linked hypogammaglobulinaemia (Bruton's disease)	Absence of B cells

SOURCE: Office of Technology Assessment, 1991; adapted from E. Gleichmann, I. Kimber, and I.F.H. Purchase, "Immunotoxicology: Suppressive and Stimulatory Effects of Drugs and Environmental Chemicals on the Immune System," *Archives of Toxicology* 63:257-273, 1989.

Chapter 3 discusses what tests are currently employed to determine whether a substance is immunotoxic and describes immunotoxic substances and their effects.

IMMUNOTOXICANTS: WHO IS AT RISK?

While exposure to immunotoxic substances can occur anywhere to anyone, the opportunity to be exposed is greater for some populations of individuals than for others. Since immunotoxic effects have been described for some ambient and indoor air pollutants, some point out that the magnitude of individuals at risk could be tremendous (21). Yet others argue that while such effects have been described for indoor air pollutants, the levels of these chemicals would generally be low—probably below the threshold for response (2).

Nevertheless, certain discrete populations are probably at increased likelihood of exposure, more amenable for scientific research, or both. For example, observations of occupational exposures to toxic substances have contributed much of the data pertinent to immunotoxicological effects—i.e., hypersensitivity, autoimmunity, and immunosuppression. Similarly, immunosuppressive drugs can be viewed as prototype immunotoxicants, and so patients receiving such drugs to prevent transplant rejection can be observed. Consumers of cosmetics have also been examined to detect hypersensitivity to various agents.

Yet even among populations of individuals, not all of those exposed are affected. Certain individuals may be more susceptible to developing adverse reactions for a variety of reasons separate from mere exposure to the toxicant—even when dose and duration of exposure are similar for different individuals. For example, the person could be innately susceptible, i.e., be genetically predisposed. Age is another important factor in immunotoxicology, since the immune system is not completely functional at birth and might be more vulnerable to damage then. It also changes appreciably in older populations—generally to a less responsive level (26).

Box 2-B—"Allergy": Some Common Misperceptions

From an immunological standpoint, the term allergy refers to a normal immune response with deleterious consequences, such as allergic rhinitis, or hay fever (e.g., to grass pollen), or contact sensitivity (e.g., to poison ivy). True "allergies" occur when a human comes in contact with a substance that triggers a predictable response that involves certain compon-ents of the immune system. Immunological hypersensitivity, or allergy, is not due to an alteration of the immune system by a foreign substance per se, but is an inappropriate activation of the immune system. Two exposures, the first involving sensitization to the substance, are needed to demonstrate allergy.

"Allergy," however, is often improperly used to denote generic adverse reactions or susceptibilities. For instance, some persons develop, or are born with, an inability to produce the intestinal enzyme lactase. These lactase-deficient individuals are unable to breakdown the sugar lactose. For them, digestion and absorption of ordinary lactose-containing milk is quite troublesome and results in gastrointestinal distress. The symptoms, however, do not arise as a result of perturbations to the immune system. Rather, the problems stem from an intolerance, not an allergy, to the milk sugar lactose.

In contrast, some problems previously thought to be intolerances might be true immune reactions, or allergies—e.g., example the condition known as celiac sprue, where an individual has an adverse reaction to wheat. In this syndrome, the soluble protein of wheat flour, A-gliadin, induces a damaging inflammatory reaction to the epithelial layer of the gut leading to a malabsorption syndrome. Although once believed to be due to another enzyme deficiency, growing evidence indicates that A-gliadin shares significant structural homology with an adenovirus protein. As a result of earlier presensitization to the adenovirus protein, epithelial lymphocytes respond to the gliadin with subsequent adverse inflammatory effects—a true immune response, and hence a true allergic response.

SOURCE: Office of Technology Assessment, 1991.

Finally, the overall state of health is also important in determining immunotoxicity.

Genetic background, in particular, is an important consideration in evaluating both potential immunotoxicity and variation in toxic effects. For example, genetic bases could account for the differential ability of individuals to produce anti-inflammatory factors, which in turn would influence how a person would be able to mount and modulate an immune response to an offending agent (8). Likewise, although almost anyone can develop an allergy to a given substance, a distinct segment (15 to 20 percent (23)) of the population is clinically atopic, i.e., individuals who are unusually reactive to a variety of substances. Several genes have been identified that could influence this hyperreactivity (16), although specifics remain to be elucidated.

Not only is there a genetic basis for controlling the immune response to a particular antigen (as determined by histocompatibility or human leukocyte antigens (HLA), which present processed antigens (3,12,17,24,29)), but other genes are responsible for certain pharmacologic abnormalities that can predispose an individual to immune dysfunctions, e.g., hyperreactivity to histamine or acetylmethylcholine. The latter substance is used to detect people with hyperreactive airways that constrict and cause wheezing when exposed to antigen. Total IgE production—central to immediate-type allergic reactions—is regulated by genes (16). Persons could have genetic differences—singular or in combination—that might predispose them to allergies to certain environmental and occupational antigens (30). Certain HLA genes also are associated with increased risk of autoimmune diseases (13,20).

Finally, in addition to intrinsic factors, external risk factors are critical. Smoking and exposure to tobacco smoke, for example, are important external risk factors that must be accounted for in assessing potential immunotoxicity in humans (6). Another potential external risk factor is the presence of coincidental pulmonary infection at the time of exposure. The association of viral (but not bacterial) infection with triggering attacks of both childhood and adult onset asthma has been documented (9). Such infections can cause bronchial hyperreactivity that may even become permanent. It has been hypothesized that such an infection occurring in a worker simultaneous with exposure to an industrial substance or environmental pollutant could result in an enhanced sensitivity to that substance. A different external factor, house dust mites, is a precondition for certain childhood asthmas (25).

SUMMARY AND CONCLUSIONS

A functioning immune system helps protect the human body from a vast array of chemical, physical, and biological foreign substances, as well as from problems that arise from within (e.g., tumor cells). Proper reaction to a stimulus requires the cooperation of several types of cells and organs, as well as feedback from other systems in the body.

Two broad types of immune responses exist: nonspecific and acquired. The latter response involves two different types of reactions: humoral immunity and cell-mediated immunity. Humoral immunity is characterized by the production of antibodies that circulate in blood and lymph fluid. Cell-mediated immunity encompasses an array of responses that involve different cell types, but does not involve antibody production.

The complexity of the immune system makes it vulnerable to the effects of toxic substances on many fronts. Immunotoxicity, then, is any alteration in an immune response—suppression, hypersensitivity, or autoimmunity—resulting from exposure to a toxic substance.

The science of immunotoxicity, i.e., immunotoxicology, is complicated by several elements: the complex nature of the immune system, age of subject, genetic background, and external risk factors, such as smoking. Genetic factors account, in some instances, for variation in immune responsiveness to an antigen, and hence need to be assessed when evaluating potential immunotoxic affects of a substance.

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Chapter 3

Immunotoxicological Tests

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INTRODUCTION

This study defines immunotoxicity as an adverse or inappropriate change in the structure or function of the immune system after exposure to a foreign substance. An overreactive or a suppressed immune system can lead to certain health effects commonly associated with immune dysfunction. Skin and respiratory allergies indicate that the immune system has become overreactive—hypersensitive—to a foreign substance. Increased rates of certain types of infection or incidence of certain tumors indicate that parts of the immune system have become suppressed. For example, researchers received an important clue regarding human immunodeficiency virus when physicians reported more frequent diagnoses of a rare infection, *Pneumocystis carinii,* and a rare tumor, Kaposi's sarcoma, in the same individual. Similarly, certain fungal or viral infections point to a suppressed immune system, for these ubiquitous infectious agents rarely surmount the body's defense systems.

Evidence accumulated over time indicates that some chemicals encountered in commerce can alter the structure or function of the immune system. Occupational and consumer experiences have identified several allergens. Clinical experience with certain drugs and studies of accidental exposure to certain industrial substances indicate that some chemicals can lead to disease by suppressing various immune functions or can cause autoimmune reactions.

Rather than wait for adverse effects to manifest themselves, chemical manufacturers and regulators now seek means to predict immunotoxicity prior to human exposure. Immunotoxicologists have developed in vivo and in vitro tests, often used in combination, to analyze the effects of substances on various components and processes of the immune system. This chapter describes methods for evaluating chemical immunotoxicity. It also provides a brief explanation of the difficulties entailed in interpreting currently available tests and using their results to predict immunotoxicity in humans. Finally, it reports on some known or suspected immunotoxicants.

TEST METHODS

The purpose of toxicity testing is to ascertain the potential for a substance to adversely affect the structure or function of an organ system. Toxicological tests must be valid, i.e., they must actually measure the effect of interest. They must also be reliable, i.e., it must be possible to duplicate the test in different laboratories with a minimal number of inaccurate results. Importantly, since the test results may be used as the basis of regulation, it must be possible to extrapolate the likely results of human exposure from data garnered from toxicological tests on experimental animals.

An immunotoxicity assessment measures quantitative and functional changes in the immune system following exposure to the test substance. It measures whether a chemical alters lymphoid organ weights or histology, causes qualitative or quantitative changes in humoral, cell-mediated, and nonspecific immunity, or modifies susceptibility to infectious agents or tumors. (Ch. 2 details the various functions of the immune system and its components.) Most tests used in immunotoxicological assessments are performed on experimental animals, usually rodents. In the best experiments, animals are exposed to a putative toxicant in a manner that mimics human exposure conditions as closely as possible. In many of the tests, tissue or fluids removed from an animal that has been killed (sacrificed) are examined in vitro to detect evidence of toxicity. Some tests, primarily the host resistance assays, are performed in vivo, but have disease or death as the endpoint measured.

Because of the harm that most current tests can do to the test subject, predictive testing for immunotoxicity using human subjects is quite rare. It is possible to perform some of the common predictive tests on human peripheral blood, bronchoalveolar lavage fluid, or nasal lavage fluid since removal of these fluids does no harm. Tests similar to those used to predict hypersensitivity are used on humans in a clinical setting—to determine the cause of allergic symptoms—but clinical testing is beyond the scope of this report.

A basic list of immunotoxicity tests and what they measure is provided in the following sections. The list is illustrative rather than comprehensive. Descriptions of basic tests and specific methodology can be found in several sources (3,58,75).

Pathology

Pathology—the science of disease—is a basic tool of toxicology. Examination of the organs and cells of the immune system using routine pathologic tests can yield evidence of immunotoxicity. Many of the following tests are applied routinely to new chemicals, and can be interpreted to suggest the need for further study of immune system effects.

Hematology—A complete blood cell count measures the total number of blood cells. A differential blood cell count discriminates between the red blood cells and the types of white cells (e.g., lymphocytes, monocytes, neutrophils, basophils, and eosinophils) in the blood. Alterations in cell counts can indicate potential immunotoxic effects.

Histology—The lymphoid organs—the thymus, spleen, lymph nodes, and bone marrow—provide information about possible immune alterations when examined at the cellular level. Changes in the thymus may indicate T cell alterations; B and T cell accumulations in specific areas of the spleen and the lymph nodes suggest a potential change in either humoral immunity (B cells) or cell-mediated immunity (T cells). Bone marrow evaluation can yield information about pluripotential stem cells (immune cell progenitors).

Organ weights—T cells complete their maturation in the thymus. The spleen contains B and T cells. Thymus or spleen weights outside normal reference ranges are viewed as important indicators of potential immunotoxicity.

Cellularity—Counts of certain cells in the tissues of the thymus, spleen, bone marrow, and peripheral lymph nodes can be used to determine potential immune alterations, since a change in cell number suggests an immunotoxic effect on that organ.

Quantitation of splenic B and T cells—B and T cells cannot be distinguished by size or shape, but they do have distinct surface markers. A variety of specialized markers can be used to identify cell types and subsets within type. Marked cells can be counted manually or by using automated cell counters. Alterations in cell counts suggest possible immunotoxic effects.

Humoral Immunity

Humoral immunity involves the production of specific antibodies by B cells following exposure and sensitization to a specific antigen (see ch. 2). The following tests of humoral immunity call for quantitation in vitro of cell types following exposure in vivo to a test agent. The tests are performed on organ cells (which come only from experimental animals) or on peripheral blood (which can come from animals or humans).

Antibody plaque forming cell (PFC) response—This test measures the number of B cells capable of producing antibodies following exposure to an antigen. The test commonly employs a T-cell-dependent antigen, such as sheep red blood cells (SRBC), and can reveal whether B cells, T helper cells, and macrophages are functioning properly. This test cannot identify the cell type (or types) responsible for the abnormal result. This assay can also be performed following immunization with T-cell-independent antigens to exclude the possibility that altera-

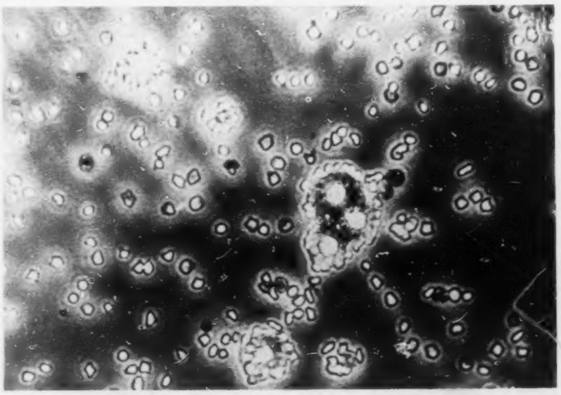

Photo credit: American Type Culture Collection, Rockville, MD

Human T cells surrounded by sheep red blood cells in a PFC assay.

tions in antibody production to T-dependent antigens are due to T helper cell dysfunction.

B cell mitogen response—This test measures the ability of B cells to proliferate (undergo mitogenesis) after stimulation by a bacterial mitogen. Analysts measure the amount of DNA synthesis by B cells from the spleen or peripheral blood after exposure to the suspect chemical. Reference values are determined in unexposed cells, and a decrease in synthesis or proliferation below those values may indicate that the B cells did not respond to stimulation.

Immunoglobulin (Ig) levels in serum—Several methods exist to measure serum or body fluid Ig levels. Changes in a given Ig level are proportional to the number of B cells secreting that particular class of antibody. The most common techniques for measuring serum Ig can identify class (e.g., IgG, IgA, or IgE) and subclass (e.g., IgG has four major subclasses, each of which can be associated with specific disorders). In the case of IgE, the radioallergosorbent test (RAST) can be used to identify antigen specific antibody.

Cell-Mediated Immunity

Cell-mediated immunity describes any immune response in which antibody plays a subordinate, rather than a dominant, role (see ch. 2). Most immune responses involve both humoral and cell-mediated immunity, but the responses can be measured separately.

The test for delayed hypersensitivity response is carried out in vivo. Other tests for cell-mediated immunity are evaluated in vitro after exposure in vivo. Human testing is possible if peripheral blood is to be evaluated, and in some cases human skin testing can be done.

T cell mitogen response—This test measures the ability of T cells to proliferate after stimulation by mitogens such as plant lectins. As with the B cell mitogen assay, analysts measure the amount of DNA synthesis in T cells from the spleen or peripheral blood. A decrease in synthesis or proliferation (below reference values) may indicate that the T cells did not respond to stimulation.

Cytotoxic T lymphocyte (CTL) assay—This assay measures the ability of certain T cells, those induced to differentiate into cytotoxic T cells, to lyse (destroy) cells of the type with which they were immunized. The cytolytic activity of these activated cells is assessed by measuring the amount of radioactivity released from radiolabeled target cells of the same type used to immunize the animal. Cytolytic activity below reference values established in the test indicates a possible immunotoxic effect.

Delayed hypersensitivity response—This assay measures the ability of the immune system to mount a delayed hypersensitivity response after injection with an antigen. Measurement of swelling and redness at the site of antigen injection can be used to evaluate the response, as can other types of assays using radioisotopic procedures that measure the influx of macrophages or serum albumin.

Mixed leukocyte response (MLR)—The MLR is a general test of cell-mediated immunocompetence. The test measures T cells' ability to recognize foreign lymphocytes, to transform and proliferate into various effector T cells, and to release cytokines.

Nonspecific Immunity

Immunity is considered nonspecific when it develops without prior contact with antigens. Tests for nonspecific immunity are most often performed on experimental animals. However, tests of natural killer (NK) cell activity can be performed using peripheral blood, and the tests measuring macrophage numbers and functions can be done using human lavage fluids.

Natural killer (NK) cell activity—NK cells are thought to lyse (kill) several types of tumor cells and virus-infected cells. The cytotoxic activity of NK cells can be measured by the amount of radioactivity released from labeled NK-sensitive targets when they are incubated together with the NK cells. The release of radioactivity is an indication that the tumor cells have been attacked and damaged in a way that would ultimately lead to their death.

Macrophages—Macrophage functions commonly measured include antigen presentation and cytokine production (mechanisms used by the macrophage for cell-to-cell communication), phagocytosis (consumption of debris and invading organisms), intracellular production of oxygen free radicals (substances to kill the or-

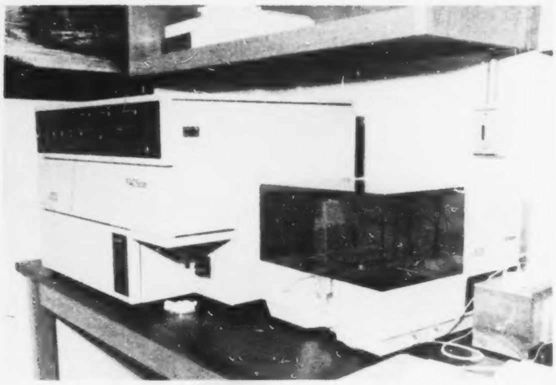

Photo credit: Medical College of Virginia, Richmond, VA

Flow cytometers are used to count cells in various immunotoxicity assays.

ganisms the cell consumes), and direct tumor-killing activities. Macrophages can also be counted in the manner described for B and T cells.

Host Resistance

Assays of host resistance – ability to fight infections or tumors – are performed on experimental animals. They test overall immunocompetence. In host resistance assays, animals are first exposed to a potential immunotoxicant and then exposed to – challenged with – a tumor, bacterium, virus, or parasite. Different components of the immune system can be evaluated by selecting specific challenge agents.

Challenge with one type of tumor cell, a PYB6 sarcoma, assesses the cytolytic activity of T cells (cell-mediated immunity) and NK cells (nonspecific immunity); challenge with cells from another tumor, the B16F10 melanoma, mainly permits evaluation of the nonspecific immunity afforded by NK cells and macrophages. Influenza virus and Streptococcus bacteria provoke antibody production and are used generally to test humoral immunity, though some scientists believe that no adequate host resistance assay of humoral immunity has been developed. Antibody apparently plays no role in resistance to Listeria bacteria, which depends on T cells and macrophages, thus Listeria challenge measures cell-mediated immunity. These challenge agents are illustrative and do not represent a complete list of tests in use.

Hypersensitivity

The assays described above generally test for a suppressed immune system. Tests for hypersensitivity can also be performed on laboratory animals to screen for a substance's allergic potential (or in an allergist's office to discern the reasons for an individual's allergic symptoms). Several types of tests have been developed to measure whether chemicals can produce an allergic response directly or by bonding with proteins in the body. Tests of skin and respiratory reactions can be performed in experimental animals and humans.

Skin reactions: Guinea pig sensitization tests—Tests to determine a substance's potential to induce delayed-type hypersensitivity – usually manifest as allergic contact dermatitis – are conducted most often in guinea pigs. The tests measure erythema and edema (redness and swelling). Common tests include the Draize test, the open epicutaneous test, the Buehler test, the Freund's complete adjuvant test, the optimization test, the split adjuvant test, and the guinea pig maximization test. The essential difference among these tests is the manner in which the animal is exposed to the test substance (3,64).

Skin reactions: Mouse tests—In addition to the guinea pig tests, a mouse ear swelling test (MEST) has recently been developed and validated. Some researchers believe the MEST is a sensitive, efficient, and cost-effective alternative to the guinea pig tests (26). More recently, a murine (mouse) local lymph node assay has been developed to identify chemicals that are contact allergens. This test assesses proliferation of T cells in the

Photo credit: Medical College of Virginia, Richmond, VA

The mouse ear swelling test.

draining lymph node of the ear after application of the chemical to the ear. The test developers find the results of the lymph node assay to be as reliable as results from guinea pig tests (47,79).

Skin reactions: Human sensitization assays—Skin tests can also be conducted in humans—either experimentally, to predict sensitization potential (on informed volunteers), or clinically, to determine the cause of an allergic reaction. Four basic types of predictive tests are used: a single induction/single challenge patch test; a repeated insult patch test; a repeated insult patch test with continuous exposure; and a maximization test. As with the animal tests, erythema and edema are measured (64). [Clinical test methods are beyond the scope of this study.]

Respiratory reactions—Experimental animals, often guinea pigs, can be tested for pulmonary hypersensitivity by measuring certain types of pulmonary function after inhalation exposure (43). Carefully controlled, clinical studies of human respiratory responses have also been undertaken (63,67,68,78).

Serum IgE levels—Total IgE levels and IgE levels specific for a particular antigen can be measured in the peripheral blood of both humans and experimental animals.

Test Selection

The time and resources available for testing new and existing chemicals generally preclude application of all tests to all chemicals. Therefore researchers try to develop screening processes that reserve the most comprehensive tests for the chemicals considered most likely to engender problems. Toxicologists often examine a chemical's structure-activity relationship to known toxicants as a preliminary step. They compare the test chemical's molecular structure to previously studied substances. If a chemically analogous substance is identified, its effects on the body—its activity—will be presumed for the test substance, to the extent the substances are similar. An evaluation of the structure-activity relationship of a new chemical can indicate whether it might affect the immune system and whether an immunotoxicity assessment is desirable. Determinations based on structure-activity relationships are not fool-proof, however, since structurally similar compounds can have quite different levels of toxicity.

Pathologic tests of immune system organs are fairly standard in general toxicologic testing. These tests can reveal structural changes in immune system organs, which can be used to determine whether additional immunotoxicity testing is warranted. The main drawback to these tests is that they assess only structure, not immune function.

Interest in screening chemicals for possible immunotoxic effects has led some chemical manufacturers and regulatory agencies to develop standardized testing tiers. Testing regimes for possible immunosuppressants are generally divided into two or more tiers. The first tier is limited to a screening-type effort intended to assess, as efficiently as possible, the integrity of the major components of the immune system. Subsequent tiers represent more indepth evaluation of those responses and also include assays that evaluate host resistance to challenge with infectious agents or transplantable tumors.

There is also some interest in developing a tier testing method for hypersensitivity assessment. One approach to assessing the potential for low molecular weight compounds to act as respiratory allergens calls for a literature search on the compound, followed by an evaluation of its ability to bind with serum proteins (required for allergenicity), followed in turn by skin tests, and, if warranted, respiratory tests (77).

Tier testing is not appropriate for all needs. FDA, for instance, eschews standardized tests in favor of a case-specific evaluation of substances intended for human consumption.

Immunotoxicology is still a developing field. Selection of immunotoxicological tests for chemicals remains discretionary. The tests applied to a particular substance are those considered most efficacious in a particular instance by the manufacturer and the regulator.

EPIDEMIOLOGIC EVALUATION OF IMMUNOTOXICITY

Environmental epidemiology, which attempts to associate disease or other adverse outcomes with an environmental exposure, measures health effects in humans at exposure conditions that are by definition realistic (4). Epidemiologic studies can identify possible associations that should be tested in laboratory environments and can be used to evaluate human health risks suggested by laboratory exposures (17).

Some epidemiologic studies of suspected immunotoxicants have been performed. Contact hypersensitivity and asthma are known hazards of many occupations because of the experience of exposed workers (29,42,57). Accidental exposure to industrial chemicals outside the workplace has occasionally yielded opportunities to study health effects in humans, as with polybrominated biphenyls (PBBs) (see box 3-A), polychlorinated biphenyls (PCBs) (see box 3-B), and dioxin (36). Some of the studies on PBBs, PCBs, and dioxin have shown evidence of quantitative immunosuppression, but none has shown evidence of actual health effects, and followup studies have sometimes contradicted the original studies. A recent study of the effects of passive smoking on children did reveal an immune-mediated effect on lung function corresponding to elevated prevalence of asthma and chest colds (12). Current epidemiologic research on immunotoxicity includes examination of the correlations between asbestos exposure and the development of immune system dysfunction (30,54,97). Some experts believe that increased immunological evaluation of asbestos workers may eventually explain the long latency between asbestos exposure and disease (14).

Epidemiologic research on any substance is complicated by the lack of good exposure data (4). Efforts to improve this situation are underway, however. In May 1990, the Board on Environmental Studies and Toxicology of the NAS and the Agency for Toxic Substances and Disease Registry cosponsored a meeting on **Frontiers in Assessing Human Exposures to Environmental Toxicants.** This meeting signaled increased emphasis on developing improved epidemiologic evaluation measures and increased coordination of Federal, academic, and industrial research efforts in this area. Confounding factors, such as diet and exposure to chemicals other than the one under study, make epidemiology a challenging field for researchers.

Idiosyncrasies of the immune system compound the difficulties for epidemiologic studies of immunotoxicants. The range of "normal" immune responses and "normal" conditions of immune system components varies greatly within the population, thus it is hard to define a "suppressed" human immune system in the absence of disease (18). Since epidemiologic studies do not permit challenging the study population with infectious agents, satisfactory evidence of immunosuppression may not be available during the study period, particularly if immunosuppression is transient.

Epidemiologic investigations of immunotoxicity are complex undertakings that require much time and many resources. Few epidemiologic studies have specifically examined environmental or occupational exposure to immunotoxic substances (33,49). They are likely, however, to be the only means of gaining realistic data on the human health effects of nontherapeutic substances.

PROBLEMS WITH PREDICTING AND INTERPRETING IMMUNOTOXICITY

The immune system is a complex toxicological target. As with other organ systems, its response to chemical insults has features that complicate the interpretation of experimental findings. For instance, test agents commonly show variations in impact on test subjects that can be attributed to species, strain, or sex differences (e.g., hexachlorobenzene stimulates the immune system in rats but suppresses the immune system in mice (103); chloral hydrate in drinking water significantly depressed humoral immune function in female mice, but male mice showed no alterations (45,108)).

Immunotoxicological studies often reveal complex dose-response relationships as well. A specific test agent can stimulate the immune system at one dose and suppress the immune system at another. Problems with interpreting dose-response relationships also manifest as problems with evaluating the immune system's reserve or redundant capacity. Scientists cannot accurately predict whether exposure to a test agent that reduces one cell type will impair immune function or whether that same test agent will stimulate another component of the immune system, which will then compensate for the impaired component (e.g., dimethylnitrosamine (DMN) reduced B and T cell function in mice, but susceptibility to Listeria was reduced because DMN stimulated macrophage function (37,108)).

Experimental conditions also influence immune response. Reaction to exposure in vivo often differs significantly from reaction to exposure in vitro (e.g., estradiol benzoate decreased NK cell activity in mice in vivo, but not in vitro (39)). Acute and chronic doses can render different results (e.g., acute doses of dichloroethane

Box 3-A—Polybrominated Biphenyls: The Michigan Case

Industrial incidents allow scientists to examine human immunotoxicity in "real-life" situations. One incident involving polybrominated biphenyls (PBBs) in Michigan illustrates some of the problems in assessing immunotoxicity in humans.

In the 1970s, a commercial preparation of PBB was accidentally used in place of an inorganic ingredient in preparing a feed supplement for lactating cows, and the supplement was subsequently used throughout Michigan in 1973 and 1974. Toxic manifestations in the cattle included reduced milk production, joint swelling, hyperkeratosis, persistent mastitis, cutaneous and subcutaneous infections, abscess formation on back legs and udder, and various reproductive anomalies, but did not involve indications necessarily related to infection. It took 9 months from initial contamination to identify the toxicant.

Many people consumed the contaminated products—milk, milk products, meat, eggs, and poultry—and PBB was found in serum and adipose tissues of individuals at least through 1980. Clinical symptoms included fatigue, a striking decrease in the ability to do physical or mental work, and an unusual requirement for sleep. Reduced memory and energy were also noted. Arthritic changes and other symptoms affecting the liver, neurological, and musculoskeletal systems were detailed. Increased susceptibility to infections or tumors, however, was not described.

Two studies, in 1977 and 1980, of farm residents exposed to PBB examined immunological parameters. These individuals were compared to age-matched controls: dairy farm residents living in nearby Wisconsin, who had not been exposed to the contaminated products, and New York City residents. Total B and T cell counts, functional mitogenic assays, immunoglobulin content, and NK cell abnormalities were studied. For at least 18 of 45 Michigan residents examined, a statistically significant decrease in the absolute number of T cells (about 60 percent of the amount in nonexposed control subjects) and a decreased mitogenic response to both T and B cell mitogens. The absolute number of B cells was unchanged. In a follow-up study, the researchers concluded that the immunological dysfunction reported in the first study had persisted, lasting at least 5 years. Elevated levels of IgG, IgA, and IgM were also reported.

Two other studies of a different, larger cohort of exposed Michigan residents, however, found no significant differences in an array of immune parameters. The first found significantly higher levels of circulating lymphocytes in the PBB-exposed group when tested in 1976 to 1977, but found no decrease in the absolute numbers of T or B cells. The second study reported no significant differences in B or T cells among exposed Michigan residents, PBB workers, and unexposed individuals. The researchers reported depressed mitogenic responses to certain mitogens in PBB-exposed individuals v. unexposed persons, but considered the values within the normal range of their laboratory, and thus did not attach any significance to the finding.

In all studies, the individuals exposed had not exhibited an unusual increase in infections or tumors, two widely accepted and applied indicators of suppression-type immunotoxicity. Although the lack of clinical manifestations might indicate that it was and is too early to predict or extrapolate immunotoxicity of PBB based on the Michigan accident, the conflicting laboratory values and conclusions of these studies, derived with different methods on different populations, underscore the problems associated with evaluating human epidemiologic studies. Clearly PBB affected many organ systems, but its *immunotoxicological* significance is difficult to discern.

SOURCES: Office of Technology Assessment, 1990 based on J.G. Bekesi, J.F. Holland, H.A. Anderson, et al., "Lymphocyte Function of Michigan Dairy Farmers Exposed to Polybrominated Biphenyls," *Science* 199:1207-1209, 1978; J.G. Bekesi, J. Roboz, A. Fischbein, et al., "Immunological, Biochemical, and Clinical Consequences of Exposure to Polybrominated Biphenyls," *Immunotoxicology and Immunopharmacology*, J.H. Dean, M.I. Luster, A.E. Munson, et al., (eds.) (New York, NY: Raven Press, 1985); J.G. Bekesi, J.P. Roboz, S. Solomon, et al., "Altered Immune Function in Michigan Residents Exposed to Polybrominated Biphenyls," *Immunotoxicology*, G.G. Gibson, R. Hubbard, and D.V. Parke (eds.) (New York, NY: Academic Press, 1983); R. Burrell, "Identifying and Controlling Immunotoxic Substances," contract paper prepared for the Office of Technology Assessment, U.S. Congress, April 1990; P.J. Landrigan, K.R. Wilcox, Jr., J. Silva, Jr., et al., "Cohort Study of Michigan Residents Exposed to Polybrominated Biphenyls: Epidemiologic and Immunologic Findings," *Annals of the New York Academy of Sciences* 320:284-294, 1979; J. Roboz, J. Greaves, and J.G. Bekesi, "Polybrominated Biphenyls in Model and Environmentally Contaminated Human Blood: Protein Binding and Immunotoxicological Studies," *Environmental Health Perspectives* 60:107-113, 1985; and J.K. Stross, I.A. Smokler, J. Isbister, et al., "The Human Health Effects of Exposure to Polybrominated Biphenyls," *Toxicology and Applied Pharmacology* 58:145-150, 1981.

Box 3-B—Polychlorinated Biphenyls: The Taiwan Case

In February 1979, rice bran oil poisoned over 2,000 people in Taiwan. The oil had been accidentally contaminated by polychlorinated biphenyls (PCBs), which were detectable in oil samples at concentrations of from 4.8 to 204.9 ppm, and in the blood of poisoned patients from 3 to 1,156 ppb. Subsequent analyses convinced most scientists that the toxic effects were associated with the chlorinated dibenzofurans (chemicals closely related to dioxin) that contaminated the PCBs. Patients experienced headaches, ocular disturbances, diarrhea, mylagia, arthritis, and general malaise.

The syndrome, which was first seen in Japan in 1968, where it is called the "Yusho Syndrome," is often referred to as "Yu-Cheng," literally "oil disease." Yu-Cheng is characterized mostly by distinctive acneiform eruptions and pigmentation, as well as other symptoms involving nonimmune organ systems. An increase in infections per se was not detailed, and infections that were reported seemed to be largely confined to superficial skin infections not usually associated with immunodeficiency.

For the most part, in vitro lymphocyte mitogen assays of patient lymphocytes showed enhanced stimulation. T cell loss was reported, particularly T helper cells, and increased skin reactivity (delayed-type hypersensitivity) to tuberculin and streptococcal enzymes was also documented. Tuberculin reactivity seemed to persist into the fourth year. These people were probably tuberculin sensitive before the accident and carried the bacteria in an inactive state. One possible future consequence of the PCB and furan exposure could be reactivation of tuberculosis, although to date this has not been reported.

SOURCE: Office of Technology Assessment, 1991; based on R. Burrell, "Identifying and Controlling Immunotoxic Substances," contract paper prepared for the Office of Technology Assessment, U.S. Congress, June 1990; Y.-C. Lu, Y.-C. Wu, "Clinical Findings and Immunologic Abnormalities in Yu-Cheng Patients," *Environmental Health Perspectives* 59:17-29, 1985; and Wilson, J.D., "A Dose-Response Curve for Yusho Syndrome," *Regulatory Toxicology and Pharmacology* 7:364-369, 1987.

suppressed the mouse immune system, but chronic administration in drinking water had no effect (62)). An oral dose can have no effect while injection of the same substance provokes a response. Thus the route of exposure can affect the tests results (e.g., as with chlordimeform exposure in mice (81)). The age or maturational status of the test animal may also affect results, with prenatal or neonatal exposure to immunotoxicants often provoking a much stronger reaction than adult exposure (e.g., as with DES (38)). Nonspecific environmental factors can also affect the immune system. For instance, even moderate dietary restrictions in mice can reduce spleen cellularity. However, the effects of cell reduction are unpredictable (e.g., the reduction in spleen cellularity was accompanied by an increase in PFC response (66,108)).

Even when toxicologists overcome the difficulties in interpreting results from tests on laboratory animals, the question remains as to whether animal responses correspond to human responses. Experience with therapeutic drugs provides evidence to support a conclusion that many results can be extrapolated from laboratory animals to humans. However, little direct evidence exists with regard to industrial or environmental exposures to immunotoxicants. Purposely exposing humans to suspected toxicants generally is considered unethical clinical practice. Epidemiologic studies have posed serious problems for researchers since reliable exposure data have usually been lacking, though there has been some improvement in this area (102).

Perhaps the greatest problem with extrapolating test results from animals to humans is evaluating the clinical significance of altered immune responses, particularly the significance of suppressing humoral, cell-mediated, or nonspecific immunity. Observational experience is based mainly on severe and long-lasting immunosuppression resulting from therapeutic drug treatments, and scientists do not know the clinical relevance of moderate and transient perturbations of the immune system. Conversely, changes in the immune system may not be immediately apparent, i.e., biologically significant changes in immune function could occur with few morphological correlates and remain subclinical until the animal or human is subjected to a particular stress or insult.

Comprehensive and reproducible testing schemes exist to evaluate immunotoxicological potential of chemicals in experimental animals. However, few chemicals have been tested in this manner, and the immunotoxicological findings in experimental animals can only serve as indicators of concern in humans. Historically, the immune system has received little attention as a target organ for toxicity, and immunology has not been an integral part of the toxicology curriculum (61). Increased education and research integrating immunology and toxicology would benefit scientists and policymakers interested in identifying and controlling immunotoxicants.

EXISTING DATA ON IMMUNOTOXICITY

Few of the chemical substances now marketed have undergone immunotoxicological testing. This section describes some of the research that has been done on substances or classes of substances to determine whether they can suppress the immune system or cause hypersensitivity or autoimmune reactions. Most of the referenced studies have been performed on laboratory animals since human studies on nontherapeutic substances are notoriously difficult. Specific note is made of the origin—animal or human—of the data.

Immune Suppression

Several substances clearly exhibit a toxic effect on the immune system by suppressing normal immune responsiveness. Table 3-1 lists several substances and classes of substances thought to be immunosuppressive toxicants. This subsection further analyzes the effects of some of these substances.

Therapeutic Drugs

Therapeutic drugs designed to prevent transplant rejection or to treat autoimmune disorders and cancer are the most thoroughly studied immunosuppressive substances. They are listed here as immunotoxicants because of the clearly demonstrated association between the therapeutic use of immunosuppressants and the increased incidence of infections and cancer (59). For instance, 50 percent of transplant patients get cancer within 10 years (65).

Table 3-1—Known or Suspected Immunosuppressants

- Halogenated aromatic hydrocarbons (HAHs)
  - PCBs
  - PBBs
  - Dioxins
- Immunosuppressive drugs
  - Azathioprine
  - Glucocorticosteroids
  - Cyclophosphamide
  - Cyclosporin A
- Pesticides
  - Organophosphates (e.g., malathion)
  - Organochlorides (e.g., DDT)
  - Carbamates (e.g., aldicarb)
- Polycyclic aromatic hydrocarbons (PAHs)
  - 3-methylcholanthracene
  - Benzo[a]pyrenes
- Benzene
- Illegal drugs
  - Cannibinoids (e.g., marijuana)
  - Phencyclidine (PCP)
  - Opiates (e.g., heroin)
- Heavy metals
  - Lead
  - Nickel
  - Cadmium
  - Mercury
  - Organotins
- Air pollutants
  - Nitrogen dioxide
  - Ozone
  - Cigarette smoke

SOURCE: Office of Technology Assessment, 1991.

The most frequently used immunosuppressive drugs fall into four basic categories—alkylating agents, glucocorticosteroids, antimetabolites, and natural products. Research remains to be done on exactly how these drugs produce immunosuppression, but each appears to act through a different mechanism. Alkylating agents disrupt cell functions, particularly mitosis. They are, therefore, highly toxic to rapidly proliferating cells, such as lymphoid cells. Cyclophosphamide is representative of this type of drug, and is used as a pretreatment in bone marrow transplant recipients to prevent graft rejection and is also used as a cancer treatment and to reduce symptoms of certain autoimmune diseases (91). Treatment with cyclophosphamide, while very effective, carries with it an increased risk of certain cancers (95).

Glucocorticosteroids alter phagocytosis and depress T and B lymphocyte function, though the exact mechanisms for these immunosuppressive effects remain unknown. Prednisolone and methylprednisolone, glucocorticosteroids, are therapeutic for transplant recipients and for individuals suffering from extreme allergic reactions (91). Glucocorticosteroids are associated with enhanced susceptibility to infection (95). Azathioprine is a widely used antimetabolite and acts chiefly by inhibiting protein synthesis (71). It is used clinically in transplant patients and also as an anti-inflammatory agent. A common side effect of azathioprine treatment is bone marrow suppression (95). Cyclosporin A, a natural product derived from fermentation products of two fungi, appears to act through modulation of mechanisms regulating immune responsiveness. Cyclosporin A suppresses T cell function but spares B cell function, and has proved successful with transplant patients, though long-term use has been shown to lead to higher infection rates and incidence of non-Hodgkins lymphoma (55).

Benzene

Benzene, a basic industrial chemical, serves as a solvent or feedstock in the synthetic chemical, printing, lithograph, rubber cement, rubber fabricating, paint, varnish, stain remover, adhesive, and petroleum industries. Benzene has been causally linked to several health problems, including immune dysfunction, and it is subject to stringent workplace regulations because of its proven carcinogenicity (1 ppm TWA; 5 ppm STEL (29 CFR 1910)). High dose exposure to benzene results in decreased immune cell function and increased lethality of infections. Acute exposure studies in rats have demonstrated bone marrow toxicity and depressed immune function. Benzene metabolites remain in bone marrow after exposure ceases, and may lead to long-term immunotoxic effects.

Workers chronically exposed to benzene (> 100 ppm) experienced increased rates of agranulocytosis and myelogenous leukemia, accompanied by an increased risk of infection (22). Depressed levels of some immunoglobulins in humans exposed to benzene have been reported (21), but a 1988 study showed no change in lymphocyte function in workers exposed for long periods to benzene concentrations of 1 to 5 ppm with peaks up to 100 ppm (107). There have been major disparities between studies of individuals that experienced short-term, high dose exposure, and studies of individuals subjected to long-term, low dose exposure. Benzene is, therefore, considered immunotoxic, but the magnitude of effect and the exposure threshold remain to be established.

Pesticides

Pesticides have been the subject of numerous animal studies of immunosuppression in recent years. Rodent studies of organophosphates, such as malathion; organochlorides, such as DDT; and carbamates, such as carbofuran, showed evidence of immunosuppression (93). However, none of these pesticides has been submitted to a systematic evaluation for immunotoxicity. In addition, it appears that intentionally added inert ingredients or other contaminants may be responsible for the observed suppressive effects (83,93). Many experts consider the animal studies indicative of potential immunotoxicity in humans, though certainly not conclusive evidence, and recommend prudence when dealing with pesticides, particularly the organochlorides, which remain stable in the environment and become concentrated in the food chain.

Most studies of the immunosuppressive effects of pesticides in workers indicate no decrease in resistance to disease even where changes in immune system components were measurable (43,48,106). However, one study of workers handling organophosphate pesticides did find an increase in upper respiratory tract infection (35). Epidemiologic evidence of the effects of pesticide exposure outside the workplace is quite sparse (23). A study of women who drank aldicarb-contaminated groundwater showed altered numbers of T cells, but the biological significance of the alteration was not demonstrated (25,93).

Halogenated Aromatic Hydrocarbons

Among the most infamous halogenated aromatic hydrocarbons (HAHs) are polybrominated biphenyls (PBB), polychlorinated biphenyl (PCBs), and dioxins. PCBs (now banned) has been used as plasticizers and as a heat transfer medium; PBB as a fire retardant. Dioxin appears as a contaminant in some commercial substances. Studies of the effects of HAHs on experimental animals indicate that they can have adverse effects on the immune system (94). Findings in laboratory animals exposed to PCBs or PBB at levels higher than most human exposures include severe atrophy of primary and secondary lymphoid organs, lower circulating immunoglobulin levels, and decreased specific antibody response (85,96). Evidence from epidemiologic studies is inconclusive regarding immunotoxicity of these substances in humans. Studies of human exposure have shown abnormal laboratory values for immune parameters, but no conclusive clinical evidence of immune aberration (see boxes 3-A and 3-B).

The name dioxin is assigned to 75 chemicals with similar composition. The most widely studied dioxin, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), affects young animals more severely than adults and leads to severe thymic atrophy and moderate atrophy of the peripheral lymph nodes at doses that cause other toxic effects as well (94). Recent animal research indicates that genetic predisposition plays a major role in susceptibility to the effects of dioxin (76,82). A study of mobile home park residents exposed to TCDD yielded abnormal in vitro test results in about 25 percent of the exposed population, but uncovered no reports of clinical disease due to demonstrated cellular immunity (36). These effects were not detectable upon reexamination of the same population, and the authors of the second paper consider the possibility that the first paper was in error (24). Some data on children accidentally exposed to TCDD show normal immunoglobulin levels and elevated lymphocyte responsiveness, but the only clinical evidence of disease is chloracne (70). The position of the majority of scientists who have examined human health effects of dioxin is that little or no harm has been done by its dissemination in the environment (31,73).

Polycyclic Aromatic Hydrocarbons

Fossil fuel combustion produces polycyclic aromatic hydrocarbons (PAHs), and distillates from these products are widespread in the chemical industry. Exposure occurs in the workplace and in the environment generally. Many PAHs are carcinogenic, and a growing body of evidence indicates that they may also be immunotoxic. A 1980 study showed that mice exposed in utero to benzo[a]pyrene (BaP), a PAH, experienced significant, persistent suppression of humoral immunity (99). A later study by the same author showed inhibition of the PFC response and the MLR when spleen cells were exposed to concentrations of BaP (100). Another PAH, 7,12-dimethylbenz[a]anthracene (DMBA), significantly increased susceptibility to murine cytomegalovirus (80). Noncarcinogenic PAHs do not appear to produce immune alterations (104). The immunotoxic effects of PAHs have not been widely studied in humans.

Oxidant Gases

The transportation system and many industrial processes produce airborne pollutants. Two air pollutants that have been studied for their immunotoxic effects are ozone and nitrogen dioxide, oxidant gases regulated under the Clean Air Act (CAA). Studies on experimental animals reveal increased susceptibility to infection after exposure to low levels of ozone and nitrogen dioxide. Pulmonary NK cell activity in rats was significantly suppressed following continuous exposure to ozone, but after 10 days, NK cell activity returned to normal, even in the continued presence of ozone (13). Dutch researchers found that ozone suppressed or enhanced pulmonary NK cell activity in the rat depending on the exposure level (101). Mice exposed to 2.5 to 5.0 ppm of nitrogen dioxide showed increased susceptibility to bacterial infection (40).

Ozone has been shown to cause immediate short-term changes in lung function and increased respiratory symptoms among healthy adults and children who exercise moderately or heavily during periods of elevated ozone concentrations. Some studies suggest that there may be some persistent effects associated with long-term exposure to ozone, although understanding of such effects, including whether they are immune-mediated, is currently limited (98). Government researchers recently summarized the results of several epidemiologic studies of nitrogen dioxide exposure (32). The studies reveal an increased prevalence of respiratory infections among individuals, particularly children, exposed to nitrogen dioxide, but small sample sizes and poor exposure data tend to lessen the significance of the findings. EPA has set national primary ambient air quality standards for ozone (0.12 ppm) and nitrogen dioxide (0.053 ppm) based on health effects other than immunotoxicity (40 CFR 50).

Indoor Air Pollutants

Some air pollutants become particularly concentrated indoors. Some of the epidemiologic evidence of the health effects of nitrogen dioxide comes from studies of individuals in homes with gas stoves. Sidestream cigarette smoke, in one study of children, increased the incidence of respiratory infections through immune-mediated mechanisms (12). Concerns have been raised about offgassing from products containing formaldehyde, but animal (20) and human (69) studies of immune functions following inhalation of formaldehyde indicate that it is not immunosuppressive.

Metals

Heavy metals are ubiquitous – in the air and water, at work and at home – and a few of them have been studied for immunotoxicity. A 1982 study of the effects of lead on macrophage function in mice indicated a significant immunosuppressive effect (46). A more recent study of lead's effects on host resistance in mice yielded conflicting results depending on time and amount of exposure, making it difficult to draw any conclusions about lead's immunotoxicity (50). The latter study also evaluated the effects of nickel and selenium. Nickel's effects on host resistance varied significantly with time and amount of exposure; selenium uniformly increased resistance to infection. Other studies of nickel indicate that it suppresses NK activity in mice and rats (86,87,89) but that nickel chloride does not adversely affect the immune system of the developing mouse (88). One study of cadmium showed that it significantly depressed NK cell activity in mice but had no effect on mortality due to infection (19). A study of cadmium in aged mice showed no immunosuppressive effects (10). The evidence for immunosuppressive effects of mercury on animals is conflicting (52). Organotins, used as heat stabilizers, catalytic agents, and antifungal/antimicrobial compounds, have been widely studied, but show highly variable effects depending on the species, times, and amount of exposure involved in the test (11,84,85,90).

Hypersensitivity

This report defines immunologic hypersensitivity as allergic response (see ch. 2). Delayed-type hypersensitivity manifests as allergic contact dermatitis and is the result of a T cell mediated inflammatory response. Immediate hypersensitivity is an antibody-mediated response that manifests as allergic rhinitis, asthma, or anaphylaxis. This section describes some problems with hypersensitivity resulting from environmental exposures.

Contact Sensitivity and Skin Disorders

The skin is an excellent route for toxic agents to enter the body. Workers engaged in leather tanning and finishing, poultry/egg processing, manufacturing sealants, adhesives, or abrasives, fish packing, boat building and repairing, and landscaping are at risk for skin manifestations of immunotoxicity called occupational dermatoses. Cell-mediated hypersensitivity causes 20 to 30 percent of occupational dermatoses (14).

Another skin disease of immunologic significance is atopic dermatitis (eczema). This condition is a chronic, periodic skin disorder, primarily of infants and children, that depends on a complex interrelationship of genetic predisposition, and an imbalance of immunologic and pharmacologic mediators in the skin (53). Environmental agents, including jewelry and cosmetics, may provoke initial attacks or recurrences (14).

A number of therapeutic and cosmetic materials provoke various types of skin reactions. Oils used as bases in many ointments for either medical or cosmetic purposes can induce contact sensitivity. If substances (e.g., perfumes) are dissolved in solvents, cutaneous absorption is greatly facilitated. One type of immunologic hypersensitivity, "underarm anti-perspirant granuloma," occurred when compounds containing the element zirconium were introduced. Although excellent antiperspirants, they induced cell-mediated hypersensitivity, which over a long period of application led to pathological manifestations of granuloma formation. Once the key ingredient was identified, the compounds were either eliminated or modified to avoid the condition.

Some common metals also cause hypersensitivity responses. The nickel in costume jewelry can cause contact sensitivity; 5 percent of all contact dermatitis can be attributed to nickel-containing compounds (14). Exposure to platinum, chromium, mercury, and gold can also lead to skin sensitization. Table 3-2 lists a number of agents known to induce types of contact sensitivity.

Respiratory Disorders

Numerous inhalants cause immune-mediated respiratory disorders, including some types (but not all) of bronchial asthma, hypersensitivity pneumonitis, allergic rhinitis, bronchopulmonary aspergillosis, silicosis, asbestosis, coalworkers' pneumoconiosis, and possibly byssinosis (60). Some of these conditions result from a humoral immune response, as in the case of IgE-mediated, bronchial asthma, while others have cell-mediated immune involvement, as with the mineral pneumoconioses.

Hypersensitivity pneumonitis is a respiratory disease of immune origin. The exact mechanism of its pathogenesis is controversial, but the involvement of several immune components, including nonspecific complement activa-

Table 3-2—Common Contact Sensitizers

- Plant
  - Poison ivy
  - European primrose
- Synthetic compounds
  - Benzocaine
  - Epoxy resins
  - Mercaptan
  - Picric acid derivatives
  - C-I Hydrocarbons
  - Ethylenediamine
  - Paraphenylenediamine
  - Thimerosol
- Minerals
  - Beryllium
  - Nickel
  - Cadmium
  - Chromates
  - Silver
  - Zirconium
  - Cutting oils

SOURCE: Office of Technology Assessment, 1991.

tion, T and B lymphocyte stimulation, and macrophage activation, has been reported (15,72). Hypersensitivity pneumonitis is caused by allergy to specific microbial or organic substances. However, the dusts that cause the condition are sometimes mixtures of many potentially inflammatory or bio-active agents.

Asthma is the leading chronic disease of childhood (27). Occupational asthma is the most common occupational respiratory ailments in the Western world (16). Numerous agents can induce asthma, and they are generally divided into two groups: large molecular weight substances, usually proteins, that cause classical, IgE-mediated asthma; and low molecular weight materials that cause non-IgE-mediated, longer lasting types of asthma. Occupational asthma is often of the non-IgE-mediated type; table 3-3 presents a list of the some common incitants for occupational asthma.

Table 3-3—Industrial Chemicals Associated With Occupational Asthma

Platinum salts	Ethylenediamine
Nickel salts	Phthalic anhydrides
Pyrethrum	Colophony resins
Diisocyanates	Exotic wood dusts

SOURCE: Office of Technology Assessment, 1991.

Substances That Are Allergenic

Some commonly used drugs cause allergic reactions in users. Penicillin produces an allergic reaction in 1 to 10 percent of users (less than 1 percent are of the life-threatening, anaphylactic variety) (1). Some drugs used to treat hypertension are also suspected of producing allergic reactions in patients using the drug for extended periods (1), although the exact mechanism of the reaction remains unknown. Over-the-counter medications are also allergenic for some users and bear warnings to that effect. It should be noted that not all adverse drug reactions are immune-mediated though the reaction may be called "allergic" (see box 2-B). For instance, aspirin produces an allergy-like reaction in some users, but the weight of the evidence now indicates a non-immunologic basis for aspirin intolerance (21).

Formaldehyde has historically been used to increase wrinkle resistance and fabric durability, and many garment industry workers were believed to develop an allergic reaction to free formaldehyde (21). However, recent challenge studies on patients with asthma yielded no evidence that formaldehyde could cause or aggravate symptoms, and attempts to measure serum antibody and skin reactions yielded no adverse reactions (69). OSHA regulates formaldehyde as a carcinogen.

Toluene diisocyanate (TDI) is used in plastics manufacture and reportedly has induced asthma and contact dermatitis in occupationally exposed individuals. Some patients with TDI-induced asthma remain symptomatic even years after cessation of exposure, and some observers believe that TDI may cause airways to become hyperreactive to agents such as smoke or air pollutants. Most individuals with TDI-induced asthma react similarly to other diisocyanates (42). OSHA now regulates TDI at levels below those demonstrated to induce hypersensitivity in humans (0.005 ppm TWA; 0.002 ppm STEL).

Definitive data demonstrate that occupational exposures to some pesticides (e.g., some carbamate and organophosphorous esters) can induce contact hypersensitivity. While animal studies indicate that antibody response to pesticide derivatives is possible (93), no reports of IgE sensitization of humans to pesticides have been confirmed. Some scientists believe reported reactions may be irritative rather than allergic in nature.

Autoimmunity

Autoimmune diseases are aberrations of the immune system resulting in an organism attacking a part of itself as a foreign substance. Certain toxic substances that are biological in nature can increase the risk of certain autoimmune conditions. For example, it is well known that certain streptococcal infections, when left untreated, may lead to rheumatic fever and post-streptococcal glomerulonephritis. However, the evidence for chemical-induced autoimmune disorders is ambiguous (2,34,41).

A growing list of pharmaceuticals have been shown to induce autoantibody formation (antibodies against self antigens) or actual autoimmune pathologies (table 3-4; box 3-C; 9,28,41). Genetic susceptibility also plays an important role in immunotoxicology and autoimmunity. Because of the strong genetic component and a generally poorer understanding of autoimmunity compared to other immune responses, deciphering the exact role of toxic chemicals in the induction of autoimmunity is difficult.

SUMMARY AND CONCLUSIONS

Scientists have developed a number of tests that assess the various components and processes of the immune system. Pathologic evaluations and certain assays of

Table 3-4—Substances Associated With Autoimmune Responses

- Antihypertensive drugs
  - Hydralazine
  - Methyldopa
- Anti-arrhythmic drugs
  - Procainamide
  - Practolol
  - Quinidine
- Anticonvulsant drugs
  - Phenytoin
  - Ethosuximide
  - Primidone
- Antimicrobial drugs
  - Penicillin
  - Sulfonamides
  - Isoniazid
  - Nitrofurantoin
- Metals
  - Lithium
  - Gold
  - Mercury
  - Cadmium
- Other substances
  - Penicillamines
  - Chlorpromazine
  - Propylthiouracil
  - Griseofulvin
  - Oxyphenisatin
  - Vinyl chloride
  - Methylcholanthrene

SOURCE: Office of Technology Assessment, 1991; based on P.E. Bigazzi, "Mechanisms of Chemical-induced Autoimmunity," *Immunotoxicology and Immunopharmacology* J.H. Dean, M.I. Luster, A.E. Munson, et al. (eds.) (New York, NY: Raven Press, 1985).

humoral, cell-mediated, and nonspecific immunity have been validated in one experimental animal, the mouse, and validation efforts are underway for other species. Tests that measure immune cell numbers have advanced more quickly than tests that measure functional immunity, but tests to evaluate specific immune functions are available.

Immunotoxicological testing—like all toxicology—presents an investigator with significant challenges. The results of tests on experimental animals often differ depending on the test subject's age, species, or sex. Environmental factors, such as diet or smoking, also affect immune system performance. Choosing the appropriate test dose and the means and duration of exposure can prove difficult when resources are limited and the point of the exercise is to extrapolate from the test to the consequences of human exposure.

Box 3-C—Chemical-Induced Autoimmunity: Spanish Toxic Oil Syndrome

Understanding of autoimmune responses to chemical exposure lags far behind hypersensitivity and immunosuppression. Following a poisoning episode in Spain, scientists made one of the few attempts to examine whether an autoimmune mechanism could explain the symptoms experienced after toxic exposure.

In May 1981, an unknown disease affecting approximately 20,000 people was reported in Madrid and northwest of the city. About 3 months after the acute phase of the outbreak, a subpopulation of individuals developed a severe neuromuscular and scleroderma-like syndrome, causing at least 350 deaths. Epidemiologic evidence supported a conclusion that adulterated rapeseed oil, sold as olive oil, was responsible for the disease.

Kammuller, et al., reported that a contaminant, 1-phenyl-5-vinyl-2-imidazolidinethione (PVIZT), was isolated in certain case-associated oil samples. Because the chemical structure of PVIZT is closely related to hydantoins and thioureylenes, which can cause autoimmune-like disorders in man, the researchers conclude that PVIZT could account for the syndrome. Patients with toxic oil syndrome presented symptoms similar to those found in known human autoimmune diseases. In addition, the researchers measured immunological changes, including high nonspecific IgE antibody levels, marked eosinophilia, decreased T suppressor cells, and several types of autoantibodies.

SOURCE: Office of Technology Assessment, 1991; based on M.E. Kammuller, N. Bloksma, and W. Seinen, "Chemical-Induced Autoimmune Reactions and Spanish Toxic Oil Syndrome: Focus on Hydantoins and Related Compounds," *Clinical Toxicology* 26(3&4):157-174, 1988.

Scientists believe that the immune system has a reserve capacity, although the size of that reserve is as yet undetermined. Thus tests that measure impairment of one immune system component may not, in fact, indicate overall immunotoxicity, since other immune components or processes may compensate for the impairment. In several of the studies cited in the preceding text, a chemical produced a discernible decrease in a specific immune function without producing a measurable decrease in host resistance to disease. Many scientists believe that without increased incidence of infection or cancer, there is no evidence of immunotoxicity. There is still much to be learned about the long-term consequences of weakening individual components of the immune system.

Immunotoxicologists have identified many substances that have demonstrable immunotoxic effects in laboratory animals, and in a few instances, the effects of these substances have been observed in humans as well. Drugs developed to control graft rejections and cancer definitely suppress the human immune system, and patients receiving these drugs provide good human data on the consequences of prolonged immunosuppression. Occupational experience (see table 3-3) has provided some evidence of substances' inadvertent immunotoxic effects in humans. Accidental exposures to suspected immunotoxicants have, in a few cases, provided the opportunity for gathering human data (see boxes 3-A and 3-B). For the most part, however, data are sparse on the effects of general exposure to immunotoxicants in the environment. The scientific community recognizes that the immune system is an important target organ for toxicity. Most scientists agree that the lack of human test data should not preclude efforts to control human exposures to suspected immunotoxicants, but the absence of data will ensure continued disagreement about suitable means and levels of control.

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Chapter 4

Federal Attention to Immunotoxicants

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INTRODUCTION

A diverse framework of laws authorizes several Federal agencies to control human exposure to toxic substances, including immunotoxicants. This chapter describes Federal research activities designed to enhance the base of knowledge about immunotoxicology and to support regulatory efforts. This chapter also provides a brief summary of the power of the Federal regulatory agencies to license, register, set standards, or otherwise control immunotoxic substances. Several previous OTA studies have described Federal programs to regulate toxic substances in much greater detail (24,26,27,28,29,30,31,32). Finally, this chapter describes Federal programs that enable workers and the general public to obtain information about the presence of toxic substances as a means to control exposure.

FEDERAL RESEARCH ACTIVITIES

Federal immunotoxicology programs focus on research, development, and validation of test methods to assess the impact of substances on the immune system. Researchers seek improved methods for assessing the toxicological bases of hypersensitivity, autoimmunity, and immune suppression. This section discusses Federal efforts to evaluate substances that may present immunotoxic health risks and to develop immunotoxicological tests for use in regulation.

The National Toxicology Program

The Secretary of the U.S. Department of Health and Human Services (HHS) established the National Toxicology Program (NTP) in 1978 to coordinate and strengthen the Department's activities in characterizing the toxicity of chemicals. NTP is charged with:

- broadening the spectrum of toxicologic information obtained on selected chemicals;
- increasing the numbers of chemicals studied, within funding limits;
- developing and validating assays and protocols responsive to regulatory needs; and
- communicating NTP plans and results to governmental agencies, the medical and scientific communities, and the public.

NTP consists of four charter agencies of HHS: the National Cancer Institute (NCI), the National Institute of Environmental Health Sciences (NIEHS), the National Center for Toxicological Research (NCTR), and the National Institute for Occupational Safety and Health (NIOSH). NTP coordinates the relevant programs, staff, and resources from those Public Health Service agencies relating to basic and applied toxicological research. An executive committee consisting of the heads of the Environmental Protection Agency (EPA), the Food and Drug Administration (FDA), the National Institutes of Health (NIH), NIEHS, the Agency for Toxic Substances and Disease Registry (ATSDR), the Consumer Product Safety Commission (CPSC), NCI, NIOSH, and the Occupational Safety and Health Administration (OSHA) oversees NTP. Nominations of chemicals for toxicology studies are made by all participating agencies and are encouraged from all sectors of the public.

The objectives of NTP's Immunotoxicology Program are to systematically: 1) evaluate and examine the influence of selected chemicals on the immune response; 2) relate alterations in immunologic functions to both general toxicity and specific organ toxicity; 3) relate changes in immunologic functions to altered host resistance; and 4) refine and employ a panel of immune and host resistance test procedures in order to better define in vitro and in vivo immunotoxicity. The Immunotoxicology Program seeks to correlate laboratory immunologic findings with altered host susceptibility and to extrapolate animal findings about chemically induced effects to estimates of human risk (17).

NTP researchers conceived a tier approach to testing for immunosuppression (12; see ch. 3). Tier I (see table 4-1) includes assays for pathology, humoral immunity,

Table 4-1—NTP's Panel of Tests for Detecting Immunotoxicity

Parameter	Procedures
Screen (Tier I)	
Immunopathology	Hematology—complete blood count and differential Weights—body, spleen, thymus, kidney, liver Cellularity—spleen Histology—spleen, thymus, lymph node
Humoral-mediated immunity	Enumerate IgM antibody plaque forming cells to T-dependent antigen(SRBC) LPS mitogen response
Cell-mediated immunity	Lymphocyte blastogenesis to mitogens (Con A) and mixed leukocyte response against allogeneic leukocytes (MLR)
Nonspecific immunity	Natural killer (NK) cell activity
Comprehensive (Tier II)	
Immunopathology	Quantitation of splenic B and T cell numbers
Humoral-mediated immunity	Enumeration of IgG antibody response to SRBCs
Cell-mediated immunity	Cytotoxic T lymphocyte (CTL) cytolysis. Delayed hyper-sensitivity response (DHR)
Nonspecific immunity	Macrophage function-quantitation of resident peritoneal cells, and phagocytic ability (basal and activated by MAF)
Host resistance challenge model (endpoints)	Syngeneic tumor cells PYB6 sarcoma (tumor incidence) B16F10 melanoma (lung burden) Bacterial models *Listeria monocytogenes* (mortality) *Streptococcus* species (mortality) Viral models Influenza (mortality) Parasite models *Plasmodium yoelii* (parasitemia)

SOURCE: M.I. Luster, A.E. Munson, P.T. Thomas, et al., "Methods Evaluation—Development of a Testing Battery to Assess Chemical-Induced Immunotoxicity: National Toxicology Program's Guidelines for Immunotoxicity Evaluation in Mice," *Fundamental and Applied Toxicology* 10:2-19, 1988.

cell-mediated immunity, and nonspecific immunity. The tests included in Tier I function as a basic immunotoxicity screening mechanism and cannot predict whether a substance will reduce the immune system's ability to fight disease. However, they can detect immune alterations that suggest the need to evaluate the compound further, using one or more of the specialized tests listed under Tier II. Tier II assays include pathologic tests and measures of humoral, cell-mediated, and nonspecific immunity, and employ host resistance challenge models that test the ability of an animal (usually a mouse) to prevent infection or tumor growth after exposure to a suspected immunotoxicant. NTP's battery of tests does not include measures of a substance's potential to induce hypersensitivity. NTP's methods cannot measure tolerance or reversibility of effect, since animals are evaluated at a single point in time, or specific sites of immune responsiveness, such as lung or intestinal immunity.

Since 1985, when validation of the NTP tiers was completed, more than 50 chemicals have been evaluated for immunosuppression (see table 4-2). NTP has also tested 2 of those chemicals and 15 additional chemicals using standard hypersensitivity assays (see table 4-3). Among the agents tested by NTP are the AIDS treatment, AZT; nitrophenylpentadien – spy dust; methyl isocyanate, the primary causative agent of the Bhopal disaster; and silicone fluid used in surgical implants.

The mouse has been the experimental animal of choice at NTP because its immune system is well characterized. Efforts are underway to validate immunotoxicity

Table 4-2—Substances Tested by NTP for Immunosuppression

Substance	Use/Industry	Immunotoxicity
acetonitrile	catalyst; solvent	–
aldicarb oxime	insecticide	–
allyl isovalerate	fragrance; flavoring agent	–
arsine	dopant for microelectronics	+
azathioprine	chemotherapeutic agent	+
benzidine	drycleaning fluid; dye manufacturing	+
benzo(a)pyrene	fossil fuel combustion byproduct	+
benzo(e)pyrene	veterinary antiseptic	–
o-benzyl-p-chlorophenol	germicide	–
t-butylhydroquinone	antioxidant in cosmetics	–
cadmium chloride	photography; dyes; lubricants	+
chemical mixture	mix of 26 groundwater contaminants	+
4-chloro-o-phenylenediamine	hair dyes; curing agent	–
cyclophosphamide	cancer therapeutic	+
2,4-diaminotoluene	photography	+
dideoxyadenosine	potential AIDS therapeutic	+
diethylstilbestrol	formerly a hormone therapy; cattle growth promoter	+
dimethylbenz(a)anthracene	induces malignant tumors (research)	+
dimethyl vinylchloride	organic synthesis	+
diphenylhydantoin	anticonvulsant therapeutic	+
ethyl carbamate	anesthetic; co-solvent; anti-neoplastic	+
ethylene dibromide	fumigant; gasoline additive	+
formaldehyde	disinfectant; tissue fixative; textiles; photography; wood products	–
gallium arsenide	semiconductors; electronics; microwave generation	+
ginseng	medicinal and research purposes	+
hexachlorobenzo-p-dioxin	chemical byproduct	+
indomethacin	analgesic; anti-inflammatory	+
interferon-alpha	cell product with antiviral activity	+
lithium carbonate	glazes; antidepressant drug	+
methyl carbamate	chemical intermediate	–
methyl isocyanate	synthesis of pesticides	–
nickel sulfate	fabrics; plating; catalyst	–
nitrobenzene	dyes; shoe polish; leather; paint; soaps	+
nitrofurazone	antibacterial agent; food additive	–
n-nitrosodimethylamine	solvent; rocket fuels; antioxidant	+
m-nitrotoluene	explosives; dyes	+
p-nitrotoluene	explosives; dyes	+
ochratoxin a	metabolite from mold	+
oxymetholone	therapeutic; synthetic androgen	–
pentachlorophenol	wood preservative	+
pentamidine isethionate	antiprotozoal used to treat pneumonia	–
o-phenylphenol	fungicide; cleaning; rubber; preservative	–
phorbol myristate acetate	tumor promoter (research)	+
ribavirin	antiviral therapeutic	+
silicone polymers	semiconductor manufacture; surgical implants	–
2,3,7,8-tetrachlorodibenzo-p-dioxin	herbicide production byproduct	+
tetraethyl lead	chemical intermediate	+
tetrahydrocannibinol	constituent of marijuana	–
4,4-thiobis(6-t-butyl-m-cresol)	antioxidant and stabilizer	+
toluene	solvent; denaturant	–
tris(2,3-dichloropropyl) phosphate	flame retardant	–
vanadium pentoxide	catalyst; glass; ceramics; photos; textiles	–
4-vinyl-1-cyclohexene diepoxide	resins	+

NOTE: Positive (+) compounds demonstrated a significant dose-response effect for any one parameter in the NTP Tiers or showed significant effects in multiple parameters at a high dose level. The designation indicates potential immunosuppresive chemicals, not definitive immunotoxicants.

SOURCE: Office of Technology Assessment, 1991; based on M.I. Luster, National Institute of Environmental Health Sciences, Research Triangle Park, NC, personal communication, July 1990.

testing in rats because they are used most frequently in general toxicological testing. The goal of toxicological testing on experimental animals is to be able to extrapolate from test results to human health effects. Thus researchers may proceed with testing and test validation in other species (e.g., dogs, swine, and primates) in order to identify the most suitable test subjects for particular immune functions.

In fiscal year 1990, NTP had a budget of almost $2.6 million for immunotoxicological research (19). NTP continues to work on refining and improving the immu-

Table 4-3—Substances Tested by NTP for Hypersensitivity

Substance	Use/industry	Immunotoxicity
benzothonium chloride	veterinary medicine	–
benzyl-p-chlorophenol	disinfectant germicide	+
4-chloro-o-0-phenylenediamine	hair dyes; curing agent	+
cobaltous sulfate	electoplating; glazes	+
crotonaldehyde	solvent; warfare	–
2,4-diaminotoluene	photography	–
dinitrofluorobenzene	reagant	+
ethylene thiourea	electroplating; dyes; rubber	+
glutaraldehyde	disinfectant; fixative	+
isobutyraldehyde	perfumes; rubber; antioxidants	–
isophorone diisocyanate	polyurethane	+
2-mercaptobenzothiazole	rubber; fungicide; oil	+
nitrophenylpentadien	spy dust	–
oleic acid diethanolamine	surfactant	–
polydimethylsiloxane fluid	water repellant; resin; surgical implants	–
triethanolamine	dry cleaning; cosmetics; textiles	–
xylenesulfonic acid	shampoos; cleaning compounds	–

NOTE: Positive (+) indicates statistically significant contact hypersensitivity response observed in mice and/or guinea pigs.

SOURCE: Office of Techology Assessment, 1991; based on M.I. Luster, National Institute of Environmental Health Sciences, Research Triangle Park, NC, personal communication, July 1990.

notoxicity test battery, and is currently engaged in reviewing data to determine whether the Tier I and Tier II assays can predict the immunotoxicity of compounds and the potential for use of the test data in risk assessment (34).

The Environmental Protection Agency

EPA's primary immunotoxicological research efforts are located in the Office of Health Research (OHR). In fiscal year 1990, OHR had 6 principle investigators engaged in immunotoxicological research and funded $345,000 of intramural research and $324,000 of extramural research (22).

EPA's research program in immunotoxicology has four primary goals:

- to develop tier testing methods in the rat similar to those used by NTP in the mouse. This effort, which is coordinated with NTP, supports guideline development for the Office of Pesticide Programs and the Office of Toxic Substances;
- to develop host resistance assays for both the mouse and rat that can be included in test guidelines, which would facilitate use of immunotoxicity testing data for risk assessment purposes;
- to develop methods for assessing immune responses in the lung (including development of appropriate host resistance models) in order to improve the ability to assess immunotoxic effects of inhaled compounds. These methods are used to evaluate National Ambient Air Quality Standard (NAAQS) pollutants as well as other compounds covered by the Clean Air Act. Such methods are needed because of the diffuse nature of the immune system and because many of the inhaled compounds assessed primarily affect the lung rather than the spleen from which cells are usually obtained for immune function tests. Effects of certain air pollutants on markers of immune dysfunction in the lung are also being assessed in human clinical studies; and
- to develop improved methods for assessing allergenic potential of compounds, including methods development in both contact sensitivity and pulmonary hypersensitivity (22).

In addition to work in the immunotoxicology program of OHR, EPA scientists have established an oral reference dose for tributyltin oxide (an antimicrobial/antifungal pesticide) based on immunotoxic concerns. The reference dose may eventually be used by regulatory offices within the agency to set exposure standards.

The Food and Drug Administration

FDA has contributed substantially to the field of immunotoxicology and held one of the first scientific conferences on inadvertent modification of immune response. Four centers of the FDA, the Center for Food

Safety and Applied Nutrition (CFSAN), the Center for Drug Evaluation and Research (CDER), and the Center for Biologics Evaluation and Research (CBER), and NCTR are currently engaged in basic immunotoxicity research and test development.

FDA uses different immunotoxic testing strategies depending on the substance tested and its intended uses, thus a standardized testing scheme is unsuited to FDA's needs. CFSAN has been involved in immunotoxicological research since the mid-1970s, when it began to develop in vitro studies to screen for potentially immunotoxic food constituents and contaminants. Current research efforts involve methods development as well as actual studies of the immunotoxic potential of food additives and contaminants. CFSAN is trying to integrate immunotoxicity with conventional toxicity testing and may soon issue guidelines for evaluating the immunotoxic potential of direct food additives (11).

Immunotoxicology research efforts at CBER are aimed at better understanding the clinical relevance of compromised immune function (5). CDER evaluates drugs on a case-by-case basis and encourages immunotoxicity testing by manufacturers where it is warranted (14). The NCTR initiated an immunotoxicity research program in 1975, and the major focus of NCTR's research program has been on development of in-vivo testing (2). FDA states that much of its workload is directly related to identifying possible toxic effects on the immune system, and the agency was unable to respond to OTA's inquiry about budget and personnel devoted specifically to immunotoxicology (3).

Other Federal Research Efforts

The NIH allocated approximately $27 million to immunotoxicological research in fiscal year 1989 (some of which went to NTP) (16). Funds for intramural and extramural research have been available at varying levels in each of the Institutes. Much of the immunotoxicity research was incidental to other research efforts, but immunotoxicology received some direct attention. For instance, the National Institute of Allergy and Infectious Diseases has supported dermatotoxicological studies of allergenic plants, assessment of how chemical additives in foods and medications can trigger asthmatic attacks, and animal studies on the causal role of workplace chemicals in asthma.

NIEHS spent approximately $7.5 million on immunotoxicological research in fiscal year 1989. NIEHS is actively involved in developing and validating immunotoxicological test methods, and provides funding for NTP's immunotoxicity testing program. NIEHS, working independently and through NTP, also performs basic research, seeking to better define the relationship between immune function changes and altered host resistance, particularly at the low end of the dose-response curve, as well as provide data that should support a framework to allow better extrapolation from animal immunotoxicity data to human health risks.

The Centers for Disease Control (CDC) also conduct immunotoxicity research. The Center for Environmental Health and Injury Control allocated $175,000 and two full-time equivalent staffers (FTEs) to a study of immunology measurements for human exposure assessment in fiscal year 1990. NIOSH conducted an assessment of immunological markers of herbicide exposure in fiscal year 1990, and provided basic support to immunotoxicological research for a total budget of over $300,000 and four FTEs. The Agency for Toxic Substances and Disease Registry (ATSDR) also provides financial support to much of CDC's immunotoxicology research (8).

The Alcohol, Drug Abuse, and Mental Health Administration (ADAMHA) carries out research to determine the effects of alcohol and abused drugs on the immune system. ADAMHA has a particular interest in the interaction between the nervous system and the immune system (6). The Department of Defense reported that it funded, in fiscal year 1990, one extramural immunotoxicity research project designed to develop a model for studying the toxicity of dioxin (15). The Department of Agriculture reported that it does not single out immunotoxic substances for research, but indicated significant research attention to aflatoxin, which has shown evidence of immunotoxicity (21).

Because of significant differences in data collection and reporting among the agencies, OTA could not arrive at an exact budget for federally supported immunotoxicological research for this background paper. Most of the agencies charged with protecting human health have some ongoing immunotoxicological research activities, much of it devoted to developing and validating tests that can be applied to substances of concern to the agencies. There is strong interest among the Federal agencies – particularly FDA, NIH, CDC, and EPA – to organize a Federal interagency committee on immunotoxicology to foster increased interaction among the agencies responsible for immunotoxicity research programs (4).

FEDERAL REGULATORY ACTIVITIES

OTA identified 12 laws with mandates broad enough to encompass immunotoxicological concerns that authorize Federal agencies to regulate toxic substances (table 4-4). None of these laws spells out a specific duty to regulate immunotoxicants, but the duty to protect human health included in each law places immunotoxicants within the regulatory reach of the administering agencies.

The Occupational Safety and Health Administration

OSHA administers the Occupational Safety and Health Act (OSH Act) of 1970 (29 U.S.C. 651 et seq.). The Act authorizes OSHA to promulgate new standards for toxic materials and to modify or revoke existing standards. Section 655(b)(5) states that:

> The Secretary, in promulgating standards dealing with toxic materials or harmful physical agents under this subsection shall set the standard which most adequately assures, to the extent feasible on the basis of the best available evidence, that no employee will suffer material impairment of health or functional capacity even if such employee has regular exposure to the hazard dealt with by such standard for the period of his working life.

Table 4-4—Major Federal Laws Controlling Toxic Substances

Act	Agency primarily responsible
Toxic Substances Control Act	EPA
Federal Insecticide, Fungicide, and Rodenticide Act	EPA
Federal Food, Drug, and Cosmetic Act	FDA
Occupational Safety and Health Act	OSHA
Comprehensive Environmental Response, Compensation, and Liability Act	EPA
Clean Air Act	EPA
Federal Water Pollution Control Act and Clean Water Act	EPA
Safe Drinking Water Act	EPA
Resource Conservation and Recovery Act	EPA
Consumer Product Safety Act	CPSC
Federal Hazardous Substances Act	CPSC
Federal Mine Safety and Health Act	MSHA

KEY: CPSC—Consumer Product Safety Commission; EPA—Environmental Protection Agency; FDA—Food and Drug Administration; MSHA—Mine Safety and Health Administration; OSHA—Occupational Safety and Health Administration

SOURCE: Office of Technology Assessment, 1991.

The Supreme Court has interpreted this language to require OSHA to enact the most protective standard possible to eliminate a significant risk of material impairment, subject to the constraints of technological and economic feasibility (*American Textile Manufacturers Institute, Inc. v. Donovan,* 452 U.S. 490 (1981)).

OSHA rulemaking can result in requirements for monitoring and medical surveillance, workplace procedures and practices, personal protective equipment, engineering controls, training, recordkeeping, and new or modified permissible exposure limits (PELs). In 1987, OSHA adopted updated standards that had been set by the American Conference of Government Industrial Hygienists (ACGIH), a voluntary organization, for workplace exposure to 428 toxic substances (52 FR 2332; 29 CFR Part 1910). Despite these new standards, OSHA lacks information on the effects of chronic exposure for over 90 percent of these substances. Most of the remaining 10 percent, which have been evaluated for chronic toxicity, have not been evaluated for immunotoxicity (36). However, an immunotoxic effect, sensitization, was specifically noted for eight of these substances (table 4-5).

Photo credit: Environmental Protection Agency, Washington, DC

Special clothing, as well as exposure limits, can help protect workers.

Table 4-5—Sensitizers Regulated by OSHA

Substance	Use/industry	Health effects
Captafol	fungicide	Skin and respiratory sensitization
Cobalt (metal, dust, and fume)	aircraft; automobile	Pulmonary sensitization
Isophorone diisocyanate	housing; automobile	Skin and respiratory sensitization
Phenothiazine	veterinary insecticide	Skin sensitization
Phenyl glycidyl ether	monomer and surfactant production	Skin sensitization
Picric acid	rocket fuels; steel	Skin sensitization
Subtilisins	laundry detergents	Respiratory sensitization
Toluene-2,4-diisocyanate	rubber; paints; coal tar	Pulmonary sensitization

SOURCE: Federal Register, vol. 53, No. 109, Tuesday, June 7, 1988.

The Food and Drug Administration

FDA regulates chemicals found in foods, drugs, and cosmetics under the Food, Drug, and Cosmetic Act of 1938 (FDCA; 21 U.S.C. 301-392). FDCA encompasses several laws passed by Congress since the first Federal statute regulating food safety, the Food and Drugs Act of 1906, including the Pesticide Chemical Residues Amendment of 1954, the Food Additives Amendment of 1958, the Color Additive Amendments of 1960, the Drug Amendments of 1962, and the Animal Drug Amendments of 1968.

Foods

FDCA declares it illegal to sell an adulterated food. A food is adulterated if:

> . . . it bears or contains any poisonous or deleterious substance which may render it injurious to health; but in case the substance is not an added substance such food shall not be considered adulterated under this clause if the quantity of such substance in such food does not ordinarily render it injurious to health (21 U.S.C. 342(a)).

FDA has authority to regulate unavoidable environmental contaminants, pesticide residues, and additives that appear in food. Added substances are governed by a stricter standard than naturally occurring substances. FDA has authority to require premarket submission of specific toxicity test data. FDA does not currently have testing guidelines for immunotoxicity in foods, but CFSAN has proposed some guidelines that are currently under review (11).

FDA regulates some substances studied as immunotoxicants, such as mercury and polychlorinated biphenyls (PCBs), based upon other adverse health effects (21 CFR Part 189). FDA regulated Yellow Dye No. 5 based on its association with hypersensitivity. FDA has also regulated the use of sulfites because they can provoke life-threatening responses—often severe asthmatic attacks—in sensitive individuals. Sulfites are no longer generally recognized as safe for use on fruits or vegetables intended to be served or sold raw or presented as fresh to consumers, or on potatoes intended to be served or sold unpackaged and unlabeled to consumers (21 CFR Part 182).

Drugs

FDCA authorizes FDA to regulate new drugs for humans and animals. The Public Health Service Act provides similar authority for biologics (e.g., vaccines, monoclonal antibodies, cytokines, and growth factors). New drugs and biologics require pre-marketing approval. In the approval process, applicants must submit two kinds of applications: 1) an Investigative New Drug (IND) application, essentially a request to conduct an investigation; and 2) a New Drug Application (NDA) or Product Licensing Application (PLA), essentially a request for permission to conduct a more detailed investigation adequate to achieve marketing approval.

The IND application must include chemical, manufacturing, and control information; pharmacologic and toxicologic information from animals and in vitro systems; and a plan of clinical study. An NDA or PLA, submitted after the research period for the IND, must include full reports of toxicological studies and clinical investigations to show that the test agent is safe and effective; a complete list of the test agent's composition; samples of the test agent; information that may be re-

Photo credit: Julios, Washington, DC—C. Onrubia

Federal regulations now prohibit the use of sulfites on salad bars because they evoke hypersensitivity reactions.

quired for monitoring; specimens of proposed labels; and information on the potential risks of inactive ingredients.

The mechanisms of immunosuppression and its clinical consequences are better understood than those of immunostimulation, due largely to experience with immunosuppressive drugs in clinical practice. FDA has approved the use of several drugs as immunosuppressive agents, such as cyclosporin A. In addition, FDA has approved drugs whose known immunotoxic effects, particularly sensitization, are outweighed by their benefits, but generally requires a warning of sensitization as a possible side effect. In CDER, each division routinely evaluates immunotoxicity as part of the total safety assessment. Many of the tests for effects on the immune system are routinely incorporated into the 28-day toxicity studies that are usually submitted as part of the IND (14). FDA's drug testing guidelines do not specifically require immunotoxicity testing, but require that an applicant convince FDA that its test data are adequate. FDA can suggest or require immunotoxicity testing where appropriate.

Cosmetics

FDA cannot, under the law, require a manufacturer to perform toxicity testing of cosmetic ingredients. However, products that have not been tested for safety cannot be marketed unless they bear a label reading "*Warning: The safety of this product has not been determined*" (21 CFR 740.10).

FDA has restricted fewer than 20 cosmetic ingredients on the finding that they were "poisonous or deleterious." Among these restricted ingredients, however, are substances known to affect the immune system, such as mercury and mercurial compounds, potent allergens and contact sensitizers (21 CFR 700.13), and vinyl chloride, a contact sensitizer (21 CFR 700.14). Despite FDA's inability to compel toxicity testing, the cosmetic and fragrance industries do operate voluntary testing programs for potential skin sensitizers (7).

The Environmental Protection Agency

EPA administers several laws that authorize regulation of toxic substances, including the Clean Air Act (CAA; 42 U.S.C. § 7401 et seq.), the Clean Water Act (CWA; 33 U.S.C. 1251 et seq.), the Safe Drinking Water Act (SDWA; 42 U.S.C. 201, 300f et seq.), the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA; 7 U.S.C. 136 et seq.), the Toxic Substances Control Act (TSCA; 15 U.S.C. 2601 et seq.), the Resource Conservation and Recovery Act (RCRA; 42 U.S.C. 6901 et seq.), and the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA; 42 U.S.C. 9601 et seq.).

The Clean Air Act

Under the CAA, EPA regulates air pollutants by setting National Primary and Secondary Ambient Air Quality Standards as necessary to protect the public health and welfare. EPA has promulgated primary National Ambient Air Quality Standards for sulfur oxides, particulate matter, carbon monoxide, ozone, nitrogen dioxide, and lead (40 CFR 50). None of these standards was based on consideration of immunotoxic effects, although ozone, nitrogen dioxide, and lead have all shown evidence of immunotoxicity in animal tests.

The 1970 amendments to the CAA also called for EPA to set standards limiting hazardous pollutants. Section 112 of the CAA authorizes EPA to set emissions standards for pollutants that may reasonably be anticipated to result in an increase in mortality or an increase in serious, irreversible, or incapacitating reversible illness. The list of substances designated by EPA as hazardous air pollutants includes asbestos, benzene, beryllium, coke oven emissions, inorganic arsenic, mercury, radionuclides, and vinyl chloride (40 CFR Part 61). Several of these substances have shown evidence of immunotoxicity in the laboratory, but serious health effects other than immunotoxicity served as the basis for these standards, which set exposure levels far below those used in the tests.

Amendments to the CAA passed by the 101st Congress (Public Law 101-549) establish a statutory list of 189 hazardous substances or classes of substances. The EPA Administrator may add or delete substances based on evidence of a pollutant's potential to cause in humans:

(i) cancer or developmental effects, or
(ii) serious or irreversible –
  (I) reproductive dysfunctions,
  (II) neurological disorders,
  (III) heritable gene mutations,
  (IV) other chronic health effects, or
  (V) adverse acute human health effects.

Photo credit: Environmental Protection Agency, Washington, DC

Few data exist on the human health risks from transient, low-level chemical exposures.

The 1990 amendments direct EPA to require application of the maximum achievable control technology (MACT) initially. Following implementation of MACT, EPA is required to evaluate residual risk from sources of these substances and decide whether public health is adequately protected; if not, stricter controls can be required.

The Clean Water Act

Since the Federal Water Pollution Control Act was first enacted in 1948, it has been amended nine times and is now generally referred to as the CWA. The 1972 amendments set the goal of achieving "fishable, swimmable" waters by 1983 and prohibiting the "discharge of toxic pollutants in toxic amounts" by 1985. The 1977 amendments endorsed a new method for regulating toxic pollutants, and the 1987 amendments continued Congress's emphasis on control of toxic pollutants.

The CWA authorizes the EPA administrator to establish and revise a list of toxic water pollutants. EPA may then issue effluent limitations or effluent standards to regulate discharges of these substances into the Nation's navigable waters. Effluent limitations, established on an industry-by-industry basis, impose technology-based restrictions on the amount of a toxic substance that can be directly discharged from a point source. Effluent standards are control requirements based on the relationship between the discharge of a pollutant and the resulting water quality in a receiving body of water. Effluent standards can be imposed when, in the judgment of the Administrator, the effluent limitations affecting a particular source are insufficient to protect the designated use of a particular water body reflected in the water quality standard for that body established by the State. This more stringent effluent *standard* is employed much less frequently than the technology-based effluent limitations.

The CWA also requires that EPA establish pretreatment standards for toxic substances discharged from private pollution sources into publicly owned water treatment facilities. In addition to these legally binding regulations, the CWA authorizes EPA to establish ambient water quality criteria for all pollutants, including toxics, to be used as water quality goals.

EPA has published a list of hazardous substances under the CWA (40 CFR 116.4) and has established reportable quantities for each of these substances (40 CFR 117.3). Under the CWA, EPA has also promulgated toxic pollutant effluent standards for six substances (40 CFR Part 129), including PCBs (known to be immunotoxic in laboratory animals). Immunotoxicity was not the endpoint of concern in these rulemaking procedures, however.

The Safe Drinking Water Act

The SDWA regulates public waters systems and addresses contaminants "which may have an adverse effect on the health of persons." Under the SDWA, EPA establishes maximum contaminant levels goals for contaminants that may have an adverse effect on health. These are nonenforceable health goals, which are used as guidelines for establishing enforceable drinking water standards. EPA then sets enforceable "maximum contaminant levels" (MCL) that are as close to the goal as feasible considering the best available technology and the economic costs of complying with the standard. MCLs have been set for inorganic chemicals (40 CFR 141.11 and 141.62) organic chemicals (40 CFR 141.12 and 141.61). Immunotoxicity was not a noted consideration in these actions.

The Federal Insecticide, Fungicide, and Rodenticide Act

FIFRA makes it unlawful to sell or distribute a pesticide that is not registered with EPA. An applicant for registration of a pesticide must file the following information with EPA: a statement of all claims made for the pesticide; directions for its use; a description of tests made upon it; and the test results used to support claims made for the substance. In addition, the applicant must supply appropriate health and safety data. EPA must register the pesticide if its composition warrants the proposed claims for it, if it will perform its intended function without unreasonable adverse effects on the environment, and if, when used in accordance with widespread and commonly recognized practice, it will not generally cause unreasonable adverse effects on the environment. An "unreasonable adverse effect on the environment" is defined as "any unreasonable risk to man or the environment, taking into account the economic, social, and environmental costs and benefits of the use of any pesticide." The burden of proof regarding safety is on the manufacturer. If EPA finds that a pesticide meets or exceeds any of its specified criteria for risk (40 CFR 154.7), it must initiate a special review process. This process allows EPA to require additional toxicity testing, including immunotoxicity, if warranted.

A pesticide may be registered for general or restricted use, and EPA may conditionally register pesticides even if some test data are unavailable. EPA has concentrated its attention to date on the active ingredients in pesticides, but expresses increasing concern about inert ingredients. EPA has issued no restrictions on pesticide use based solely on immunotoxicity. The 1988 amendments to FIFRA require EPA to review 600 active ingredients of existing pesticides by 1997, which requires reexamination of safety, including toxicity. The test guidelines for reregistration are the same as for registration.

In 1982, EPA's Office of Pesticide Programs (OPP), which administers FIFRA, published data requirements for the toxicological evaluation of biochemical pesticides and for microbial pesticides. Biochemical pesticides include pheromones, hormones, natural insect and plant growth regulators, and enzymes. Microbial pesticides include bacteria, fungi, protozoa, and viruses. OPP recently revised the requirements to reflect advances in toxicology (23). The immunotoxicity study now required for biochemical pesticides is designed to accommodate either the rat or the mouse as the test animal. The tiered testing scheme as revised is presented in table 4-6. The study is required for biochemical pesticides where uses result in significant human exposure (e.g., food uses, indoor aerosols). Tier I tests serve as a screen for immunotoxic potential, and Tier II tests are designed to provide information necessary to perform risk assessment. Tests to determine whether biochemical pesticides can induce a delayed-type hypersensitivity reaction in guinea pigs are required and are set forth as a separate study in the data requirements (40 CFR 158.690).

The revisions of the data requirements deleted the requirement for specific immunotoxicity testing of microbial pesticides, but the ability of the test animals to clear the active microbial ingredient after dosing via oral, pulmonary, and intravenous routes is used as an indicator of a properly functioning immune system. EPA reserves the right to require an immunotoxicity study for certain microbial pesticides, but this study would be reserved for certain viruses that are related to viruses known to impact adversely on the human immune system (23). The requirement for a hypersensitivity assessment of

Table 4-6—EPA Subdivision M Guidelines: Proposed Revised Requirements for Immunotoxicity Testing of Biochemical Pest Control Agents

Tier I

A. Spleen, thymus, and bone marrow cellularity
B. Humoral immunity—do one of the following:
   1. Primary and secondary immunoglogulin (IgG and IgM) responses to antigen
   2. Antibody plaque forming cell assay
C. Specific cell-mediated immunity — do one of the following:
   1. One-way mixed lymphocyte reaction (MLR) assay
   2. Effect of BPCA on normal delayed-type hypersensitivity
   3. Effect of BPCA on generation of cytotoxic T-lymphocyte
D. Nonspecific cell-mediated immunity:
   1. Natural killer cell activity
   2. Macrophage function

Tier II

A. Tier II studies required if:
   1. Dysfunction is observed in Tier I tests
   2. Tier I test results cannot be definitively interpreted
   3. Data from other sources indicate immunotoxicity
B. General testing features:
   1. Evaluate time-course for recovery from immunotoxic effects
   2. Determine whether observed effects may impair host resistance to infectious agents or to tumor cell challenge
   3. Perform additional specific, but appropriate, testing essential for evaluation of potential risks

SOURCE: Office of Technology Assessment, 1991.

microbial pesticides also has been dropped, with reporting of any observations of allergic reactions being required instead. This is because it is expected that proteinaceous components of microbial pesticide preparations (including fermentation byproduct ingredients) would elicit a positive response in test guinea pigs after subcutaneous induction and challenge; and would most likely not give a positive response with topical induction and challenge.

OPP plans to revise its testing guidelines for chemical pesticides to include immunotoxicity testing. Laboratory studies presently required for registration of chemical pesticides include a battery of acute toxicity studies (oral, dermal, pulmonary, eye), subchronic studies, chronic studies, developmental toxicity studies, reproduction effects study, battery of mutagenicity studies, chronic carcinogenicity study, and metabolism study (23).

The Toxic Substances Control Act

TSCA authorizes EPA to regulate chemicals (specifically excluding pesticides; tobacco and tobacco products; nuclear materials; foods, drugs, and cosmetics; pistols, firearms, revolvers, shells, and cartridges, which are regulated under other statutes) before and after they reach the market. EPA's first task under TSCA was to compile an inventory of all existing chemical substances that would be subject to the provisions of TSCA that were manufactured or imported into the United States in 1977. Any chemical not on that initial list is a "new" chemical and subject to premanufacture notice (PMN) requirements.

TSCA requires manufacturers to notify EPA in advance of the intended introduction into commerce of a new chemical with a Premanufacture Notice (PMN). EPA must also be notified if a chemical is to be used in a way that differs significantly from that proposed in the original PMN. The PMN contains data on a chemical's identity and structure, proposed use, manufacturing byproducts, and impurities.

TSCA does not require that manufacturers carry out a specific program of toxicity testing before approval of a new chemical, thus PMNs are rarely submitted with toxicity data for each organ system. The extent of toxicity data submitted with PMNs generally depends on the projected annual production volume for the compound. If insufficient or incomplete toxicity data are provided to support a PMN, EPA requests additional information or issues a consent order in which the manufacturer agrees to provide the required information according to an established timetable.

EPA toxicologists evaluate PMNs by comparing new chemicals to structurally related existing chemicals. If toxicity is predicted on the basis of structural analogues, a chemical may be subjected to a more detailed examination. If during the detailed review EPA concludes that a new chemical may present an unreasonable risk of adverse effects on human health or the environment, additional toxicity data can be required. Immunotoxicity has not been used as the basis for any regulatory action taken by EPA under the PMN provisions of TSCA.

TSCA also directs EPA to regulate existing chemical substances that pose an unreasonable risk of injury to human health or the environment and to act promptly on substances that pose imminent hazards. An Interagency Testing Committee reviews substances on the existing chemicals list and can recommend that EPA require testing for these substances. In determining whether a chemical presents or may present an unreasonable risk to human health or the environment, EPA considers:

- the effects of a substance or mixture on human health and the magnitude of the exposure of human beings to it;
- the effects of a substance or mixture on the environment and the magnitude of the exposure of the environment to such substance or mixture;
- the benefits of such substance or mixture for various uses and the availability of substitutes for such uses; and
- the reasonably ascertainable economic consequences of the rules, after consideration of the effect on the national economy, small business, technological innovation, the environment, and public health.

If EPA can show that there is inadequate information on the effects of a chemical and that testing is necessary to obtain that information, it may issue a test rule defining the substances to be tested and how they should be tested. EPA has developed general guidelines for toxicity testing (40 CFR 796), but each test rule contains requirements specific to the chemical under scrutiny. Chemical manufacturers and processors are responsible for developing these test data, but EPA bears the burden of proof in establishing that a substance is an unreasonable risk to human health or the environment.

For either new or existing chemicals, EPA regulatory efforts may include steps to: prohibit their manufacture, processing, or distribution in commerce; limit their uses or amounts; require certain labeling; require maintenance of records and monitoring; prohibit or regulate any manner or method of commercial use; prohibit or regulate their disposal. Manufacturers or processors are required to notify EPA of any unreasonable risks posed by new or existing chemicals. Immunotoxicity has been a noted concern in evaluations of chemicals under TSCA, but has not served as the health effect of primary concern in any regulatory action.

The Resource Conservation and Recovery Act

RCRA defines solid and hazardous wastes, authorizes EPA to set standards for facilities that generate or manage hazardous waste, and mandates a permit program for hazardous waste treatment, storage, and disposal facilities. Hazardous waste is defined as any solid waste that may cause death or serious disease, or may present a substantial hazard to human health or the environment if it is improperly treated, stored, transported, or disposed of. Lists of wastes subject to RCRA regulation can be found at 40 CFR 261.31, .32, and .33. The list contains known immunotoxicants, but immunotoxicity has not been the basis for any chemical's appearance on this list.

The Comprehensive Environmental Response, Compensation, and Liability Act

CERCLA (sometimes referred to as Superfund) requires anyone who releases significant amounts of hazardous substances into the environment to notify EPA. CERCLA defines hazardous substances as substances identified as toxic by the CWA, RCRA, CAA, or TSCA, and any substance which "when released into the environment may present substantial danger to the public health or welfare or the environment." CERCLA also requires that hazardous waste sites be cleaned up to a standard that ensures the protection of human health and environment. Reportable quantities (RQ) were set for each hazardous substance on the basis of aquatic toxicity, mammalian toxicity, ignitability, reactivity, chronic toxicity, and potential carcinogenicity. Immunotoxicity has been a consideration but not a primary factor in setting RQ standards.

Other Federal Regulatory Activity

EPA, FDA, and OSHA exercise the main regulatory authority over toxic substances, including immunotoxicants. Other agencies also administer laws that could be used to control these substances, however. The Consumer Product Safety Commission (CPSC), for instance, enforces the Consumer Product Safety Act (CPSA) (15 U.S.C. 2051 et seq.) and the Federal Hazardous Substances Act (FHSA) (15 U.S.C. 1261 et seq.).

The CPSA authorizes regulation of consumer products (except for foods, drugs, and cosmetics; pesticides; tobacco and tobacco products; motor vehicles; aircraft and aircraft equipment; and boats and boat accessories) that pose an "unreasonable risk" of injury or illness. CPSC may set safety standards that specify requirements for product performance or design, requirements for consumer instructions or warnings, or both. A product can be banned if adequate safety standards are not feasible. No products have been regulated under CPSA on the basis of immunotoxicity.

The FHSA covers hazardous substances (excluding pesticides, foods, drugs, cosmetics, certain radioactive materials, and tobacco and tobacco products) in general use in the home, and is meant particularly to protect children from hazardous toys and products. A hazardous substance is a substance or mixture that may cause substantial personal injury or substantial illness as a proximate result of any customary or reasonably foreseeable handling or use, including reasonably foreseeable ingestion by children. A product can be required to bear a hazard label or it can be banned if labeling is inadequate to protect health. No products have been regulated under FHSA on the basis of immunotoxicity.

The Mine Safety and Health Administration (MSHA) regulates the exposure of miners to toxic substances under the Federal Mine Safety and Health Act Amendments of 1977 (30 U.S.C. 801 et seq.). Much of MSHA's regulation of toxic exposures involves incorporating by reference the lists of ACGIH. Some observers question use of the standards set by ACGIH since they historically have been set without reference to adequate research (36). On the other hand, as demonstrated by the very few standards that have been enacted by OSHA, hardly any workplace chemical exposures would be regulated if the ACGIH standards were not adopted by MSHA and OSHA (28).

FEDERAL INFORMATION PROGRAMS

Some Federal programs incorporate the assumption that an informed public is one means to decrease toxic exposures. Worker Right-to-Know programs, established by OSHA's hazard communication standard, and Community Right-to-Know programs, established by the Emergency Planning and Community Right-to-Know Act of 1986 (EPCRA), require that workers and other citizens be provided with knowledge about the toxic substances in their work or local environment. The Federal Government funds a national database at the National Library of Medicine that helps distribute information collected under EPCRA nationwide. Federal law also established the Agency for Toxic Substances and Disease Registry (ATSDR), which maintains a nationally available list of toxic substances and their health effects. The following section briefly describes each of these programs.

Worker Right-to-Know

In 1983, OSHA first established its hazard communication standard (29 CFR 1910.1200). This standard requires each employer to have a written hazard communication program for each workplace, including a list of all hazardous chemicals in the workplace. The employer is permitted to rely substantially on manufacturers and importers of chemicals to prepare the necessary information.

There are four basic elements of a hazard communication program. First, each manufacturer or importer of a chemical must determine whether that chemical is hazardous. A health hazard is defined as a chemical for which there is "statistically significant evidence based on at least one study conducted in accordance with scientific principles that acute or chronic health effects may occur." Second, each manufacturer or importer must prepare a material safety data sheet (MSDS) containing comprehensive information on the chemical, including all its hazards, precautions for safe handling and use, and control measures. These MSDS must be available to employees and customers. Third, employers must label containers to alert workers to the identity and significant hazards of the chemical. Finally, each employer must provide its workers with education and training in the handling of hazardous chemicals (13,20).

The standard requires disclosure of immunotoxic effects, where known. The standard does not permit OSHA to compel testing to determine unknown health effects, however. It should be noted that many MSDS contain very limited information on known toxic hazards (32), and those hazards that are described may be expressed in terminology unintelligible to the lay public (10).

Community Right-to-Know

Congress enacted EPCRA (42 U.S.C. 110001-11050) in response to releases of chemicals at Bhopal, India. EPCRA requires EPA to establish and maintain a list of "extremely hazardous substances." The current list includes 420 substances set out in 40 CFR Part 355 Appendix A. EPA has developed a threshold planning quantity (TPQ) for each substance on the list.

This law requires owners and operators of facilities that store, use, or release extremely hazardous substances in amounts in excess of the TPQ to report to EPA information about those chemicals, their amounts, and their locations. EPCRA requires facility owners and operators to report releases of these chemicals into the environment whether from accidental spills or normal operations. The statute does not limit use or release of a substance; it merely requires that the public be informed (9).

Local community organizations must be notified of any offsite spills or any releases of a "reportable quantity" (RQ) of an extremely hazardous substance or a hazardous substance as defined in CERCLA. The RQ's for extremely hazardous substances are set out in 40 CFR Part 355, Appendix A. The list of hazardous substances and their RQ's under CERCLA are set out at 40 CFR 302.4. This emergency notification must include the chemical's common name, the lists on which it appears, the quantity released, the time and duration of the release, the media into which the release occurred, any acute or chronic health risks presented by the release, precautions to be taken, and the persons to contact for further information (1).

EPCRA community right-to-know provisions also require the public availability of material safety data sheets similar to those prepared under OSHA's hazard communication standard. The MSDS must contain the chemical and common names of the chemical, the chemical's physical and chemical characteristics, its physical and health hazards, its routes of exposure, precautions and emergency response procedures, exposure limits, and possible carcinogenic effects. If immunotoxic effects are among the known health hazards, they must be listed (35). Some research indicates that the MSDS, which were developed to convey information about workplace exposures, are unsuited to a community information program and that better means to communicate information about risk to the general public are needed (10).

National Library of Medicine: Toxicology Information Program

The National Library of Medicine (NLM) is the Nation's principal resource for the collection, organization, and retrieval of scientific literature in the health and biomedical fields (25). It has provided data about toxic chemicals and their hazards to the public for over 20 years. To enhance the accessibility of this information, NLM established the Toxicology Data Network (TOXNET). This database contains several files, including the Toxic Chemicals Release Inventory (TRI), which contains data on the estimated releases of toxic chemicals to the air, water, or land, as well as amounts transferred to waste sites. Current law requires U.S. industrial facilities to report the TRI information to EPA, which in turn provides it to NLM for public access. Searches of this file can be performed by region, company, chemical, but the file does not contain information on the health effects of, or human exposure to, these chemicals.

Another TOXNET file, the Hazardous Substances Data Bank (HSDB), covers chemical toxicity, as well as emergency handling procedures, environmental fate, human exposure, detection methods, and regulatory requirements. This file contains information on 4,200 chemicals. Most of the data in this file is taken from peer-reviewed journals. TOXNET also includes the Registry of Toxic Effects of Chemical Substances (RTECS), which covers 100,000 chemicals, and contains information on their acute and chronic effects, carcinogenicity, mutagenicity, and reproductive consequences. This file is not peer reviewed.

NLM also maintains the TOXLINE group of databases, outside TOXNET, which contains references to journal articles dealing with hazardous chemicals and other areas of toxicology and environmental health. At present, interested parties must contact a health science library or information center to request a TOXLINE search. In the future, more public libraries may tie in to NLM. Individuals can request an application form to use NLM's system on a personal computer (33,18).

The Agency for Toxic Substances and Disease Registry

Congress created ATSDR in 1980, and its mission is to prevent or mitigate adverse human health effects and diminished quality of life resulting from exposure to hazardous substances in the environment. As part of its mission, ATSDR prepares toxicological profiles for hazardous substances which are most commonly found at facilities on the CERCLA National Priorities List and which pose the most significant potential threat to human health.

Each toxicological profile must include:

- an examination, summary, and interpretation of available toxicological information and epidemiological evaluations on the hazardous substance;
- a determination of whether adequate information on the health effects of each substance is available or in the process of development; and
- an identification of toxicological testing needed to identify the types or levels of exposure that may present significant risk of adverse health effects in humans.

ATSDR also has a Division of Health Education which coordinates health communication and education activities for the Agency; coordinates development and educational activities for emergency response personnel; develops and disseminates to physicians and the health care providers materials on the health effects of toxic substances; establishes and maintains a list of areas closed or restricted to the public because of contamination with toxic substances; and initiates research. In addition, ATSDR has regional staff, located throughout the United States, who offer consultation on environmental health issues, including emergency response.

SUMMARY AND CONCLUSIONS

The Federal Government is actively involved in advancing the state-of-the-art of immunotoxicology. EPA, FDA, and NIH have immunotoxicological research programs, and each of these agencies contributes to the work of the NTP. Much of the ongoing Federal research is directed toward developing and validating tests for evaluating substances for immunotoxic potential. NTP has published a panel of tests for immunotoxicity testing that has been validated in the mouse. NTP continues to work on validating immunotoxicity tests in other species and on improving its current panel of tests. NTP is also applying the tests to various substances. EPA is working on developing and validating immunotoxicity tests using the rat as the test species, and has published immunotoxicity testing guidelines for certain pesticides. Immunotoxicity is a major concern of FDA researchers when evaluating new products for human and animal consumption.

Few substances have been regulated by the Federal Government on the basis of immunotoxicity. OSHA has issued regulations for eight substances on the basis of their ability to provoke hypersensitivity. FDA has restricted the use of Yellow Dye No. 5 and sulfites in foods because of their association with hypersensitivity. These agencies and EPA regulate other substances that have shown evidence of immunotoxicity in a few animal tests, but other health effects serve as the basis for those regulations.

Several Federal activities are designed to enhance public awareness of the hazards of toxic substances, including immunotoxicants. OSHA's hazard communication standard requires that workers be provided with information about known health hazards in their jobs. However, since so little information is available regarding immunotoxic effects, and since the standard cannot be used to compel testing, the standard does little at present to protect workers from immunotoxic hazards. Community right-to-know legislation requires EPA to collect information about substances that pose potential toxic hazards to local communities and make that information available to the public. As with the OSHA standard, however, this program does not permit EPA to require that health effects information be developed, therefore available information on immunotoxicity is very limited. ATSDR is disseminating information about health risks, including immunotoxicity, to the public.

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Appendices

Appendix A

Income Replacement for Individuals Disabled by Immunotoxicants

INTRODUCTION

Even the best efforts to prevent harmful exposures to toxic substances are imperfect. When regulations or other precautions fail to prevent toxic exposure, permanently or temporarily disabling illnesses sometimes result. Federal and State level programs have evolved to provide a continuing source of income for disabled individuals. This appendix presents a brief overview of Social Security, State workers' compensation programs, and toxic tort. The initiatives described in this appendix were not specifically designed to compensate individuals for exposure to toxic substances, but they are available, within certain limits, to immunotoxicant exposure victims.

SOCIAL SECURITY BENEFITS

If an illness or injury interferes with a person's ability to work and lasts, or is expected to last, for more than a 12-month period, it can give rise to full Social Security benefits, either disability benefits (SSDI; 42 U.S.C. 423) or Supplemental Security Income (SSI; 42 U.S.C. 1381-1383a). To secure these benefits, a claimant must prove inability to work. It is not necessary for a Social Security claimant to prove that illness or injury occurred because of a work-related incident.

SSDI benefits are available to persons who would otherwise be qualified for Social Security benefits had they paid into the system for the requisite number of quarters (as established by 20 CFR 404.130). The SSI program guarantees a minimum level of cash income to needy aged, blind, and disabled persons who may not otherwise be eligible for Social Security.

A Social Security claimant files an application with the Social Security Administration (SSA), which then turns it over to the State administering agency. The State assesses the claim and medical evidence presented against a standard set by law, which defines disability as the inability to do any substantial gainful activity by reason of any medically determinable physical or mental impairment which can be expected to result in death or which has lasted or can be expected to last for a continuous period of not less than 12 months (4).

For purposes of SSDI and SSI benefits, a medically determinable diagnosis of an immune-system injury—including an injury or illness caused by an immunotoxicant—would be compensable. A diagnosis should be supported by a medical history, clinical findings, laboratory findings, and information regarding treatment and prognosis. Well known immune system disorders, such as a severe allergy, asthma, or autoimmune disease (e.g., rheumatoid arthritis) could be medically determinable to result in a disability.

More problematic for Social Security claimants and the SSA is the issue of environmental illness. In its Program Operations Manual System (POMS), SSA now recognizes the claim of some medical practitioners that exposure to toxic substances can damage the immune system, and the SSA identifies this claim as environmental illness (POMS 24515.065, Evaluation of Specific Issues-Environmental Illness). In the POMS, the SSA states that while there is no evidence that claimants with this type of claim have immune deficiency, immune complex disease, autoimmunity, or abnormal functioning of their immune systems, evaluation should be made on an individual case-by-case basis to determine if the impairment, whether or not immune system related, prevents substantial gainful activity. Some legal and medical practitioners claim that this standard should increase claimants' success in obtaining Federal benefits after exposure to immunotoxic substances.

WORKERS' COMPENSATION

Workers' compensation laws vary among the States, but all laws share the requirement that the injury or illness occur on the job and that the claimant be temporarily or permanently disabled as a result. This section briefly describes worker's compensation programs and concludes with a short summary of some workers' compensation claims based on damage to the immune system, and a synopsis of recent congressional interest in workers' compensation.

Basic Workers' Compensation Law

State workers' compensation programs represent a compromise reached 80 years ago between labor and business, before many of the chemicals in common use today were developed or their hazards understood. Under the common law, the basic duty of employers was to act with due care for employee safety, as a reasonably prudent person would, and to furnish a sufficient number of safe tools and equipment, as well as a sufficient number of qualified employees to do the work. Employers were responsible for issuing and enforcing rules for workplace safety, rules that with ordinary care would prevent reasonably foreseeable accidents. Finally, employers had a duty to warn workers of unusual hazards.

In theory, if the employer failed to live up to this standard of conduct, an injured employee could sue for damages under the common law. This was seldom easy, however. The first difficulty was simply proving the employee's case. Other employees might be crucial witnesses, but when few governmental or union job protections existed, anyone who testified against an employer risked being fired. The common law also established three powerful defenses that employers could use against lawsuits brought by employees: negligence of other servants or co-workers; knowledgeable assumption of risk by the employee; and contributory negligence by the injured employee. Just prior to enactment of the workers' compensation statutes, however, some States began to ease the claimant's burden of proving employer negligence, and a few workers began to win sizable judgments (16).

Workers' compensation substituted a regular, fixed, and predictable compensation payment, previously unavailable to most workers, for uncertain, potentially ruinous liability judgments, which were becoming uncomfortably frequent for employers. The first State law to withstand challenge passed in the State of Wisconsin in 1911; all States had worker compensation laws by 1948. Initially workers' compensation laws only covered accidental injury and excluded occupational disease. However, all States covered occupational disease by 1967.

Three basic approaches to occupational disease coverage have been adopted. States generally elect either to:

- establish a schedule of covered diseases (which minimizes problems of proof but excludes unlisted diseases);
- establish a schedule and a residual clause that allows claims for unlisted disease to be made, with the burden of proof on the claimant; or
- cover diseases "peculiar to" or "characteristic of" relevant trades.

Most States exclude coverage of the ordinary diseases of life (even where risk of ordinary disease may be notably increased by an occupation) and require that a worker face a hazard greater than that to which the general public is exposed.

Disease claims that are readily connected to workplace exposure and are relatively inexpensive (e.g., acute dermatoses) are compensated like accidental injuries. Disease claims involving serious disabilities that are less clearly linked to workplace exposures (e.g., chronic respiratory disease) are marked by extended controversy and long waiting periods between the time a claim is filed and a decision on that claim. For these claims, the system retains many of the undesirable features of the tort system that workers' compensation was supposed to supplant.

Some observers believe that workers' compensation systems have failed to keep pace with knowledge of the hazards of chemical agents (9,11). According to OSHA, occupational diseases from chemical exposures represent a continuing, complex problem for workers' compensation programs. Disabilities resulting from occupationally induced illness often are less clearly defined than those from occupationally induced injury. As a result, OSHA finds that workers' compensation is often a weak remedy in the case of occupational disease (52 FR 26843). This finding is used as partial justification for OSHA's decisions to regulate certain chemical exposures rather than rely on market-based incentives or other Federal programs to ensure worker health and safety.

Disease claimants face extended conflict over the issue of causality, in part due to meager scientific evidence to show a relationship between exposure and disease. The fact that many years often intervene between exposure and disease also plays a role (19). In addition, proof of causality may be ambiguous where cases are aggravated by lifestyle choices (e.g., smoking) or other non-workplace exposures to hazardous substances. Some States have opted for a statutory presumption that a claimant's disease is work-related, but that is not the prevalent approach. One study found that 60 percent of occupational disease claims are contested, and 15 percent of challenged claims result in an award for the worker (9).

Occupational disease now accounts for about 1 percent of all workers' compensation claims. The insurance industry expects the number of occupational disease claims to rise significantly during the 1990s if medical research more clearly shows a link between illness and workplace exposure to harmful substances (18). Thus the results of future research on immunotoxicity could greatly affect the number and success of workers' compensation claims based on exposure to immunotoxicants.

Even if a claimant establishes a right to workers' compensation, the amount collected may not be perceived as adequate recompense for the illness. In a majority of States, the maximum amount paid a temporarily disabled worker is at least 100 percent of the State's average wage, but 22 States pay less than that. Table A-1 shows the maximum weekly benefits paid by each State and the percentage of the State's average wage this amount represents. These temporary disability benefits are paid in addition to medical expenses, but obviously represent a substantial decline in income for many workers (18). While this problem is not peculiar to claims based on exposure to toxic substances, it may serve to discourage claims that have less than a certainty of success, like toxicant-caused illnesses, since the rewards for the claimant and a legal representative are relatively low.

Immunotoxicity Claims

The legal literature includes some published cases of workers' compensation awards to individuals claiming an immune system disorder following exposure to a toxic substance. In *Grayson* v. *Gulf Oil Company*, 357 S.E. 2d 479 (South Carolina Court of Appeals, 1987) total disability benefits were awarded under South Carolina's workers'

Table A-1—State Workers' Compensation Disability Benefits, 1989

The first column shows the maximum weekly benefit paid to workers whose disability is total but temporary. The second column shows that payment as a percentage of the State's average weekly wage.

	Maximum weekly benefit	Maximum benefit as percentage of State's average weekly wage
Alabama	357.98	104
Alaska	700.00	130
Arizona	276.15	74
Arkansas	209.08	67
California	224.00	51
Colorado	371.28	94
Connecticut	671.00	144
Delaware	280.64	71
District of Columbia	513.00	102
Florida	362.00	102
Georgia	175.00	47
Hawaii	358.00	101
Idaho	193.80	60
Illinois	604.73	142
Indiana	274.00	73
Iowa	684.00	207
Kansas	271.00	77
Kentucky	343.02	100
Louisiana	267.00	75
Maine	471.83	143
Maryland	407.00	102
Massachusetts	444.21	103
Michigan	409.00	92
Minnesota	391.00	100
Mississippi	206.60	68
Missouri	289.75	78
Montana	318.00	103
Nebraska	245.00	78
Nevada	368.82	99
New Hampshire	600.00	162
New Jersey	342.00	75
New Mexico	283.70	85
New York	300.00	63
North Carolina	376.00	110
North Dakota	313.00	102
Ohio	400.00	102
Oklahoma	231.00	66
Oregon	388.99	108
Pennsylvania	399.00	103
Rhode Island	360.00	100
South Carolina	334.87	102
South Dakota	289.00	103
Tennessee	252.00	72
Texas	238.00	61
Utah	347.00	101
Vermont	544.00	161
Virginia	393.00	105
Washington	389.32	102
West Virginia	367.89	103
Wisconsin	363.00	100
Wyoming	354.00	99

SOURCE: U.S. Department of Labor, based on laws in effect on July 1, 1989.

compensation statute on the basis of petrochemical hypersensitivity, which led, according to the claimant's physician, to a cascade of dysfunction of the claimant's immune system. The treating physician wrote that, "... the constant exposure over 19 years to the petrochemicals in her workplace dysregulated [the claimant's] immune system resulting in an allergic or hypersensitivity cascade to her total environment, including all foods, chemicals, and her own microbiological flora." The court found that the plaintiff's hypersensitivity was created by her workplace and left her unable to function properly in any environment.

A Florida appeals court also upheld a workers' compensation award based on an immune injury. In *Dayron Corporation* v. *Morehead* 509 So. 2d 930 (Florida 1987), the claimant developed a permanent sensitivity to a coolant used in his workplace. Although the claimant showed no signs of illness when outside of the workplace, the sensitivity was deemed a permanent disability for purposes of entitlement to workers' compensation. Workers' compensation benefits were also awarded in *Kyles* v. *Workers' Compensation Appeals Board* (California Court of Appeals, 1st Appellate District, Division 4, 1st Civil No. AO37375, Oct. 21, 1987), where long-term PCB exposure was found to have led to chemical hypersensitivity.

These cases indicate that courts will entertain a claim for workers' compensation based on an immune injury if the claimant establishes a work-related cause. Some States, most notably California, are considering an insurance system that would compensate employees regardless of the work-relatedness of their illness or injury, but such plans remain in a very preliminary stage at this time.

The cases also indicate, however, that such claims often proceed to court after they fail to be settled at the administrative level. This increases the costs of the system and delays workers' receipt of benefits. If the inability to work extends for a prolonged period, workers can quickly become impoverished. Most State workers' compensation statutes also foreclose a worker's ability to bring a tort case against the employer unless the worker can reasonably claim that the employer intentionally caused the injury or illness.

Congressional Interest in Workers' Compensation

Congress has been concerned with the workers' compensation system for many years. In 1970 Congress created a National Commission on State Workmen's Compensation Laws, which concluded, in a 1972 report, that State laws in general were "inadequate and inequitable." The Commission urged Congress to impose national standards if the States did not act quickly on the Commission's key recommendations. On average, States now comply with just over 12 (out of 19) of these recommendations (18). No Federal standards have been enacted.

Hearings were held in 1979 on the National Workers' Compensation Standards Act, which would have guaranteed minimum compensation levels nationwide and created an advisory panel to look at causation issues. Then Secretary of Labor Raymond Marshall, and several other witnesses, testified to the peculiar problems of compensating occupational disease, especially issues of causation and long latency (24). Hearings were also held in 1981 and 1983 on bills that would have created Federal standards of compensation for work-related exposure to toxic substances (21,22). None of those bills was enacted. No bills introduced in the 101st Congress attempted to reform the workers' compensation system as it relates to toxic exposures.

TORT CLAIMS

This section briefly describes how tort law provides compensation for injuries due to exposure to toxic substances. It also summarizes a few cases based on evidence of immune system damage, and briefly discusses recent congressional activity related to tort law.

Basic Tort Law

Tort law is part of the common law system. Its purpose is to compensate persons injured as the result of the conduct of another. In order to receive compensation for an injury through the tort system, a claimant, or plaintiff, files suit alleging tortious (wrongful) conduct on the part of the defendant. To prove a case, the plaintiff must show that he or she has suffered damage, that the damage was caused by the defendant, and that the defendant had no sufficient justification or legal right to cause this damage (13). If the plaintiff convinces the jury (or the judge in a non-jury trial) that the defendant was more likely than not the cause of the plaintiff's damages, compensation is awarded to the plaintiff.

Claims for damages based on exposure to hazardous substances are called toxic torts. As part of the required proof, the plaintiff must show that the defendant caused him or her to be exposed to a toxic substance, that the substance is capable of causing the type of damage suffered by the plaintiff, and that the particular exposure caused by the defendant was, more likely than not, the cause of plaintiff's damage. Toxic tort cases are difficult to prove in general, and present special difficulties when immune-system impairment is claimed since immunotoxicology has only recently been developed, some of its methods remain controversial, and agreement has not been reached about what constitutes immune system damage.

Immunotoxicity Claims

Cases involving damage to the immune system began appearing in the early 1980s and have increased in the intervening years. Some attorneys now estimate that hundreds, and perhaps thousands, of cases incorporating claims of immune system damage now stand in State and Federal court systems. Since the immune system is intimately connected with the body's capacity to respond to foreign substances, a large range of cases exist for which a claim of immune damage may be relevant.

Despite the difficulties of proving a case, several tort suits have claimed damages from exposure to immunotoxicants. OTA found the following cases in published databases. These cases are not presented as an exhaustive listing of immunotoxicity case law, but as illustrative of the circumstances in which individuals can be exposed, the damages alleged, and the compensation recovered.

It should be noted that many of the plaintiffs in these cases claim to have a disease or syndrome commonly referred to as multiple chemical sensitivity (MCS). MCS is a poorly understood and controversial phenomenon. Its proponents claim that exposure to certain chemicals, even at very low levels, can create immunological, neurological, and other problems for its sufferers. Its opponents argue that no scientific evidence supports such claims. Proponents and opponents agree that additional research will be required to prove or disprove the existence of MCS and its cause or causes. This background paper does not attempt to weigh the merits of claims by MCS proponents or opponents and presents these cases solely because of their use of immune system evidence.

CASE: *Woodrow Sterling et al.* v. *Velsicol Chemical Corp.*, 647 Fed. Supp. 303 (W.D. Tenn. 1986); affirmed in part, reversed in part, 855 F 2d 1188.

Claim: Plaintiffs (community residents) claimed that immune system injury accounted for present disorders (including pulmonary disease, respiratory problems, seizures, and learning disabilities) and increased the future risk of developing disease. Immune system injury was claimed to have been caused by exposure to various chemicals, including carbon tetrachloride and chloroform, in well water contaminated by Velsicol.

Evidence: Immunological tests consisted of a white blood cell count, a lymphocyte count, a total T cell count, a B cell count, a null cell count, and a breakdown of the T cell count into T helper and T suppressor cell counts. (See chapters 2 and 3 for descriptions of the immune system and immunotoxicological tests.) Plaintiffs' experts testified that the data was consistent with a diagnosis of chemically induced immune dysregulation.

Outcome: The trial court awarded damages of $75,000 to four of the five plaintiffs for impairment of the immune system and $500,000 to the fifth plaintiff, a child. The appeals court reversed the district court's award of damages for immune system impairment, finding that the plaintiffs' experts' opinions were insufficient to sustain the burden of proof. The award for immune system damage constituted only a portion of the total award ($5,273,492 in compensatory damages; $7,500,000 in punitive damages; interest on compensatory damages at 8 percent annually). The case has now been settled (5).

CASE: *Elam* v. *Alcolac*, 765 S.W. 2d 42, 4 TXLR 167 (W.D. Mo. Ct. of Appeals)

Claim: The plaintiffs (community residents) claimed that immune system injury was partially responsible for numerous adverse health conditions. Injuries were attributed to exposure to toxic waste originating in an Alcolac chemical plant.

Evidence: Tests of immunological parameters included a mitogen challenge, total T cell counts, total T helper and T suppressor cell counts, and natural killer cell counts. The plaintiffs' expert witness testified that each of the 31 plaintiffs was suffering from immunosuppression.

Outcome: The jury awarded a $49 million verdict to the plaintiff for claims including immunotoxicity. The trial court judge set aside this verdict; an action affirmed by the appeals court (which found that the term "chemical AIDS" used in association with the plaintiffs' condition was inflammatory). The case was then settled out of court for an undisclosed amount (25).

CASE: *Barth* v. *Firestone Tire and Rubber Co.* ,673 F. Supp. 1466 (N.D. Cal. 1987).

Claim: The plaintiff (a worker at the Firestone plant) claimed that he suffered injury to his immune system and onset of diseases in their latency stage as a result of Firestone's fraudulent concealment of hazardous substances at the workplace and lack of required safety devices and protective clothing. He also claimed emotional distress. The plaintiff alleged exposure to benzene, heavy metal compounds, and other industrial chemicals used in the manufacturing of tires. Plaintiff sought creation of a medical monitoring fund as remedy for the class.

Evidence: The case has not reached the trial stage and no evidence has been presented.

Outcome: The Federal district court denied a motion by defendant to dismiss the claim on grounds that there was no legally cognizable the injury. The California Superior Court has ruled that the class is unascertainable (17).

CASE: *Moore* v. *Polish Power, Inc.*, 720 S.W. 2d 183 (Tex. Ct. App. 1986).

Claim: The plaintiff (who purchased a carpet from the defendant) claimed neurological and muscular problems as a result of exposure to formaldehyde off-gassing from Polish Power's carpet.

Evidence: The plaintiff's expert witness testified that the plaintiff suffered from damage to her immune system from exposure to formaldehyde, which led to the neurological and muscular problems.

Outcome: The trial court excluded evidence from the medical expert witness relating to characteristics, formaldehyde content, formaldehyde emission rate, and dangerousness of carpet and carpet pad based on his lack of expertise on the chemistry of carpets. The court allowed the expert's opinion that formaldehyde was cause of carpet buyer's physical problems. The jury brought in a verdict for Polish Power. The Court of Appeals reversed the trial court's exclusion of the carpet evidence, and the case was sent for a new trial on the merits.

CASE: *Higgens* v. *Aerojet-General Corp.*, 1986 Env't Rep. (BNA) 1183 /Nos. 287147, 290449, 290450 (Cal. super. Ct. 1986).

Claim: The plaintiff alleged immune system damage, among other injuries, resulting from defendant company's disposal of tricholoroethylene (TCE) and other solvents in unlined ditches on his property.

Evidence: Plaintiff's experts testified that the plaintiff suffered from immune system damage, basing their findings on a blood sample from the plaintiff. Defendant's expert countered that since there was no base line measurement, the blood tests were inconclusive. Two immunologists testified for the defendant that the medical community had not accepted the immune dysfunction theory as valid. The defendant's experts also testified that, according to the immune dysfunction theory, the plaintiff was constantly being exposed to immunotoxic substances and that it was impossible to say that a particular exposure was "more probable than not" to be the cause of a given injury (12).

Outcome: Aerojet received a jury verdict in its favor. The case was not appealed, and the parties reached a settlement (8).

CASE: *Stites* v. *Sudstrand,* 660 F. Supp. 1516 (W.D. Mich. 1987).

Claim: Plaintiffs (community residents) claimed increased risk of cancer and emotional distress, partially supported by evidence of immune system injury, due to improper disposal of TCE, which was claimed to have entered drinking water.

Evidence: The plaintiffs' expert testified that the plaintiffs suffered damage to their immune systems and dysfunctions of a major enzyme system, and to his belief that those two problems resulted in a "greatly increased susceptibility to a number of future illnesses, particularly cancer." Defendants countered with an affidavit from 9 experts in immunology, stating that they could not show to a reasonable certainty that the plaintiffs would develop cancer.

Outcome: The court issued a summary judgment for the defendants on the claim for increased risk of cancer, finding that none of the plaintiff's experts were able to quantify enhanced cancer risk. The court also ruled, however, that claims for damages for fear of cancer could go to the jury. The case was eventually settled for an undisclosed amount (6).

CASE: *Lowe* v. *Norfolk & Western Railway Company,* 463 N.E.2d, 792.

Claim: Forty-seven plaintiffs (railroad employees) alleged various physical ailments, including immunological damage, arising from exposure to dioxin contained in a chemical, orthochlorophenol, which was spilled while being transported by the defendant.

Evidence: The plaintiffs' expert testified that tests performed on blood samples to evaluate the body's immune system indicated that each plaintiff showed some abnormality of the immune system.

Outcome: The jury returned a verdict in favor of the plaintiffs totaling $57,965,000. The appeals court reversed the verdict on the basis of errors in trial procedure and remanded the case for a new trial. The case was then settled for an undisclosed amount (15).

Congressional Interest in Toxic Tort

Congress has occasionally considered enacting legislation directed specifically to compensating victims of non-work-related toxic exposures. The Toxic Tort Act of 1979 would have created an independent agency within the EPA to compensate victims of pollution related injuries regardless of fault (in addition to creating a Federal cause of action for victims of toxic substances) (24). During the 98th Congress, legislation was considered in the House of Representatives that would have provided compensation for injury, illness, or death resulting from exposure to hazardous substances (23). Some Members of Congress have argued that such legislation was an implicit promise of the Superfund legislation, which requires environmental cleanup, but no bills have been enacted and none was considered in the 101st Congress, though general product liability reform legislation was proposed and debated.

DIFFICULTIES COMMON TO PROVING WORKERS' COMPENSATION OR TOXIC TORT CLAIMS

Workers' compensation was intended to be a no-fault system of compensation—if employment causes an illness or injury, the worker should recover. Tort is a system largely based on fault, where it generally must be proved that the defendant breached a duty owed to the plaintiff. Only employees can collect workers' compensation; tort is available to everyone except employees covered by a workers' compensation system. Despite these significant dissimilarities, claimants under either system share the common burden of proving causation. It is not sufficient for claimants to show that they have suffered an injury, they must show that the workplace or defendant caused the injury in order to collect compensation or damages. This section discusses some of the difficulties entailed in proving that exposure to an immunotoxicant caused a particular disorder.

Scientific Uncertainty

A commonly made claim of immunotoxicity, which is sometimes referred to as "chemically induced immune dysregulation," is that exposure to a chemical or substance impairs the body's immune system, thereby rendering an individual hypersensitive to chemicals and/or more susceptible to many ailments, including cancer. Many scientists doubt whether state-of-the-art immunotoxicology can actually establish immune system dysfunction as a result of chemical exposure. For instance, there is no agreed upon definition of "normal" immunological parameters. As discussed in chapter 3, scientists do not know how great a quantity of any particular cell type is required for proper immune function. Cell counts can vary greatly among individuals that appear to have functional immune systems.

It is also the case that various environmental and host factors, such as exposure to other toxic and nontoxic chemicals, tobacco, alcohol, drugs, or radiation, cooking habits, bacteria, viruses, nutritional imbalances, obesity, and existing medical conditions, may provide alternative explanations for plaintiffs' conditions. The illnesses from which immunotoxicity plaintiffs allegedly suffer generally do not have chemical-specific pathologies and occur in the general population. It is, therefore, difficult to isolate the specific cause of such alleged findings and illnesses in light of the panoply of environmental and host factors, and to prove causation with the degree of certainty required by law (3,14).

Warring Experts

The plaintiff must rely on scientific and medical experts to establish that he or she has suffered some type of physical damage and that the toxic substance is capable of causing that damage. Experts may also be required to prove that the defendant caused the plaintiff to be exposed to the toxic substance and to attest to the extent of the plaintiff's damages. These expert witnesses must convey their highly specialized knowledge to the trier of fact (the judge or jury) who generally has little scientific training.

Most often, experts who present evidence of immunotoxicity for the plaintiff are clinical ecologists. These medical practitioners are at odds with much of the established medical and scientific community. The American Academy of Allergy and Immunology has published position statements refuting the theory that any valid scientific evidence supports the theory that exposure to chemicals or pollutants in the environment adversely affects the function of T cells and rejecting the medical effectiveness of treatments prescribed by clinical ecologists (1,2). This disagreement among experts adds to the difficulty of trying immunotoxicity claims since judges and juries have difficulty sorting out the scientific evidence.

Continuing Debate Over Animal Testing

Very few substances have been tested to determine whether they are immunotoxic. Where testing has been done, it has generally been done on animals. Human evidence is available from clinical trials or case reports concerning immunosuppressive therapeutics. Very few epidemiologic studies have been conducted on immunotoxicants, and the results have been inconclusive. Recent court cases, however, have found plaintiffs' experts' opinions regarding immunotoxicity based on animal data unsupported by epidemiologic data to be inadequate to sustain the burden of proof (7,10). This absence of data presents a serious dilemma for plaintiffs.

Further scientific developments in the field of immunotoxicology should eliminate much of the scientific uncertainty and, presumably, end many of the disagreements between clinical ecologists and the rest of the medical and scientific community. The necessity of animal testing is an ongoing debate in U.S. society, and resolution lies well outside the field of immunotoxicology (20).

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Appendix B

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NOTE: OTA is grateful for the valuable assistance and thoughtful critiques provided by the reviewers and contributors. The views expressed in this background paper, however, are the sole responsibility of the Office of Technology Assessment.

Appendix C

Glossary of Terms and Acronyms

Glossary of Terms

Acquired immunity: Disease resistance acquired after birth and characterized by antigen-specific processes. *See* HUMORAL IMMUNITY and CELL-MEDIATED IMMUNITY; *Compare* NONSPECIFIC IMMUNITY.

Adaptive immunity: *See* ACQUIRED IMMUNITY.

Agranulocytosis: Absence of granulocytes in the blood.

Allergen: A substance known to be an agent contributing to hypersensitivity.

Allergy: Immunologic hypersensitivity. *See* HYPERSENSITIVITY.

Anaphylaxis: Acute reaction that follows rapid introduction of an antigen into an individual having preexisting IgE antibodies. Systemic anaphylaxis develops within seconds and is characterized by contriction of the larynx and bronchi and falling blood pressure. Local anaphylactic reactions are acute inflammatory reactions caused by local contact with antigen. *See* IMMEDIATE HYPERSENSITIVITY.

Antibody: A protein produced by B cells in response to stimulation by an antigen and that reacts specifically with that antigen. Antibodies are immunoglobulins.

Antigen: A substance that brings about an immune response when introduced into the body. Antigens are usually high molecular weight compounds, such as proteins. However, low molecular weight compounds (e.g., drugs or industrial chemicals) can bind to serum proteins and become antigenic.

Asbestosis: Fibrosis of the lungs resulting from inhalation of asbestos fibers.

Asthma: A usually chronic condition characterized by episodes of labored breathing.

Atopic: Having a tendency toward immediate hypersensitivity reactions due to IgE antibodies. Approximately 10 percent of the population manifest one or more forms of atopy. See IMMEDIATE HYPERSENSITIVITY; ANTIBODY.

Autoantibody: An antibody against a self antigen. *See* ANTIBODY.

Autoimmunity: A condition characterized by cell-mediated or humoral immunologic response to antigens of one's own body. This occasional departure from the usual recognition of self and nonself contributes to a variety of diseases.

B cell: A lymphocyte that produces antibodies. *See* LYMPHOCYTE; ANTIBODY.

Bronchoalveolar lavage fluid: The fluid obtained from the lungs by lavage. Lavage is a technique in which an organ is flushed with water in order to analyze material in the drainage fluid (in this case, cells from the bronchioles and alveoli).

Bronchopulmonary aspergillosis: Infection of the bronchi and lungs by the species *Aspergillus*, characterized by inflammatory lesions.

Bronchus: One of the large conducting air passages of the lung.

Byssinosis: An occupational respiratory disease associated with inhalation of cotton, flax, or hemp dust. It is characterized initially by chest tightness, shortness of breath, and cough but may lead to permanent lung damage.

Carcinogen: A substance that causes cancer.

Cell-Mediated immunity: Immunological reactions initiated and mediated by T cells. *See* T CELL. *Compare* HUMORAL IMMUNITY.

Challenge: Administration of an antigen to assess the state of immunity. In immunotoxicological testing, an experimental animal is **challenged** with an infectious agent or tumor cells to determine whether exposure to a chemical decreased the animal's ability to fight infection or cancer. *See* ANTIGEN.

Complement: A series of reactions involving approximately 20 proteins that circulate in the blood in an inactive form. When the first complement substance is triggered – usually by an antibody locked to an antigen – it sets in motion a ripple effect. As each component is activated, it acts upon the next in a precise sequence of carefully regulated steps. This phenomenon, known as the complement cascade, causes release of the chemicals that produce the redness, warmth, and swelling of the inflammatory response. It can also bring rapid death to bacteria and other cells by puncturing their cell membranes. See ANTIBODY; ANTIGEN.

Contact sensitization: To stimulate an immune response upon initial skin contact with an antigen with the consequence of preparing the body for a stronger response upon reexposure to the antigen.

Cytokine: A substance produced by cells, including T cells, that transmits messages between cells to control and modulate immune response.

Cytotoxic: Lethal to cells.

Delayed-type hypersensitivity: An inflammatory reaction that occurs 24 to 48 hours after challenge with antigen and is a result of cell-mediated immunity. *See* HYPERSENSITIVITY.

Dermatitis: An inflammatory skin condition.

Dose-response: The quantitative relationship between exposure to a substance, usually expressed as a dose, and the extent of toxic injury or disease.

Edema: Swelling.

Endpoint: The disease, condition, or adverse effect resulting from exposure to a toxic substance (e.g., immunosuppression, infection, cancer, death).

Epidemiology: The scientific study of the distribution and occurrence of human diseases and health conditions and their determinants.

Erythema: Redness.

Hematology: The science of blood and its nature, function, and diseases.

Histocompatibility: The extent to which individuals or their tissues are immunologically similar.

Histology: The study of the minute structure, composition, and function of body tissues.

Host resistance: The ability of an organism to mount a successful immune response against disease-causing antigens.

Humoral immunity: Immunity associated with antibodies that circulate in the blood. *See* ANTIBODY. *Compare* CELL-MEDIATED IMMUNITY.

Hypersensitivity: A state of heightened reactivity to a previously encountered antigen.

Immediate hypersensitivity: Immune response mediated by antibodies, characterized by hives, wheezing, and/or abrupt changes in blood pressure, and occurring with a few minutes or hours after exposure to an antigen.

Immune system: A specialized group of body cells, cell products, tissues, and organs that respond to foreign organisms and substances in the body.

Immunize: To deliberately introduce an antigenic substance (vaccination, or active immunization) or antibodies (passive immunization) into an individual, with the aim of producing immunity to a disease. Immunotoxicologists sometimes refer to the process of exposing an animal to an antigen in order to test the animal's ability to mount, at some later point, an immune reaction to the antigen as **immunization.**

Immunocompetence: The capacity to respond immunologically to an antigen.

Immunoglobulin. Protein that has antibody activity or that is antigenically related to an antibody. They are grouped into five categories based on structural differences: IgG, IgM, IgA, IgD, IgE. *See* ANTIBODY.

Immunology. The science concerned with the phenomena that allow an animal to respond to a subsequent exposure to a foreign substnce in a way that is distinct from the way it responds to the initial exposure to that same substance.

Immunologic: Pertaining to immunology.

Immunosuppression: Suppression of immune response.

Immunotoxic. Having the potential to adversely affect immune response or damage components of the immune system.

Immunotoxicant: A substance that elicits an adverse immune response or damages the immune system.

Immunotoxicity: An adverse or inappropriate change in the structure or function of the immune system after exposure to a foreign substance.

Inhalant: A substance that may be taken into the body through the respiratory system.

Innate immunity: *See* NONSPECIFIC IMMUNITY.

In vitro: Literally, in glass; pertaining to a biological process taking place in an artificial environment, usually a laboratory.

In vivo: Literally, in the living; pertaining to a biological process or reaction taking place in a living organism.

Latent effect: A reaction to a toxic substance that is not immediately evident but that appears later in life; also referred to as a silent effect.

Leukocyte: A white cell. Major classes of leukocytes are granulocytes, lymphocytes, and monocytes.

Lymphocyte: A specialized leukocyte involved in the immune response. B-lymphocytes originate in the bone marrow and when stimulated by an antigen produce circulating antibodies; *See* HUMORAL IMMUNITY. T-lymphocytes are produced in the bone marrow and mature in the thymus gland and engage in a type of defense that does not depend directly on antibody attack; *See* CELL-MEDIATED IMMUNITY. *See* B CELL; T CELL.

Lymphoid organs: The principal organs of the immune system, including bone marrow, thymus, spleen, and lymph nodes. They produce, store, and distribute the immune system cells.

Lymphokine. A protein that mediates interactions among lymphocytes and is vital to proper immune function. Interleukins and interferons are lymphokines. Lymphokines are cytokines.

Lyse: To break up or rupture a cell membrane.

Macrophage: A type of large, amoeba-like cell, found in the blood and lymph, which ingests dead tissue, tumor cells, and foreign particles such as bacteria and parasites. The macrophage also plays an important role in antigen processing and presentation.

Mitogenesis: The initiation of cell division, or mitosis.

Monocyte: Phagocytic, large leukocytes containing one nucleus.

Myelogenous leukemia: A cancer of the blood granulocytes that usually occurs in persons of age 30-50.

Nasal lavage fluid: The fluid obtained from the nasal passages by lavage. *See* BRONCHOALVEOLAR LAVAGE FLUID.

Natural killer cell: A type of lymphocyte that attacks cancerous or virus-infected cells without previous exposure to the antigen. Also called NK cell.

NK cell: *See* NATURAL KILLER CELL.

Nonself: That which is not recognized by an individual's immune system as being a natural constituent of that individual's body.

Nonspecific immunity: Immunity that exists from birth and that occurs without prior exposure to an antigen; also called innate immunity. *Compare* AQUIRED IMMUNITY.

Pathology: The scientific study of the cause of disease and of the associated structural and functional changes that are the result of disease.

Peripheral blood: Blood in the circulation remote from the heart.

Pesticide: A generic term referring to toxic substances developed to control pests; they include insecticides, fungicides, rodenticides, and herbicides.

Phagocytosis: Consumption of foreign particles (e.g., bacteria) by cells that use ameboid movement to surround the particle and then ingest it. Macrophages are phagocytes.

Pneumoconiosis: A condition characterized by the deposition of mineral dust in the lungs as a result of occupational or environmental exposure.

Pneumonitis: Inflammation of the lungs.

Reference dose (RfD): A term used to characterize risk and derived by applying safety factors to the highest level at which a substance produces no effect. If human exposure to a substance is below the RfD, no risk is assumed to exist; if exposure exceeds the RfD, risk is assumed to exist. The term may be used interchangeably with acceptable daily intake.

Rhinitis: Inflammation of the lining of the nose.

Right-to-know laws: State and local laws requiring companies to inform workers and communities of the chemical names and hazards of their products.

Self: That which is recognized by an individual's immune system as being a natural constituent of that individual's body.

Silicosis: A condition of lung fibrosis which is brought about by prolonged inhalation of silica dust.

Structure-activity relationship: The relationship between a chemical's structure and the biochemical changes it induces.

T cell: A lymphocyte produced in the bone marrow that matures in the thymus and is integral to cell-mediated immunity. T cells regulate the growth and differentiation of other lymphocytes and are involved in antibody production. *See* LYMPHOCYTE.

Teratogen: A substance that causes physical defects in offspring by adversely affecting developing embryos.

Toxicity: The quality of being poisonous or the degree to which a substance is poisonous.

White cell: A colorless cell in the blood, lymph, or tissues that is an important component of the immune system. *See* LEUKOCYTE.

Acronyms

ACGIH	– American Conference of Governmenal Industrial Hygienists
ADAMHA	– Alcohol, Drug Abuse, and Mental Health Administration
AIDS	– Acquired Immune Deficiency Syndrome
ATSDR	– Agency for Toxic Substances and Disease Registry
AZT	– Azidothymidine, now known as zidovudine
CAA	– Clean Air Act
CBER	– Center for Biologics Evaluation and Research (FDA)
CDC	– Centers for Disease Control
CDER	– Center for Drug Evaluation and Research (FDA)
CERCLA	– Comprehensive Environmental Response, Compensation, and Liability Act
CFSAN	– Center for Food Safety and Nutrition (FDA)
CPSA	– Consumer Product Safety Act
CPSC	– Consumer Product Safety Commission
CTL	– cytotoxic T lymphocyte
CWA	– Clean Water Act

DES – Diethylstilbestrol
DHHS – Department of Health and Human Services
DMN – Dimethylnitrosamine
DNA – Deoxyribonucleic acid
DTH – Delayed-type hypersensitivity
EPA – Environmental Protection Agency
EPCRA – Emergency Planning and Community Right-to-Know Act
FDA – Food and Drug Administration
FDCA – Food, Drug, and Cosmetic Act
FHSA – Federal Hazardous Substances Act
FIFRA – Federal Insecticide, Fungicide, and Rodenticide Act
FTE – full-time equivalent
HAH – halogenated aromatic hydrocarbon
HLA – human leukocyte antigen
HSDB – Hazardous Substances Data Bank
Ig – immunoglobulin
IND – Investigative New Drug
LPS – lipopolysaccharide
MCL – maximum contaminant level
MCS – multiple chemical sensitivity
MEST – mouse ear swelling test
MLR – mixed leukocyte response
MSHA – Mine Safety and Health Administration
NAAQS – National Ambient Air Quality Standards
NCI – National Cancer Institute
NCTR – National Center for Toxicological Research (FDA)
NDA – New Drug Application
NIEHS – National Institute of Environmental Health Sciences
NIH – National Institutes of Health
NIOSH – National Institute for Occupational Safety and Health
NK – natural killer
NLM – National Library of Medicine
NTP – National Toxicology Program
OHR – Office of Health Research (EPA)
OPP – Office of Pesticide Programs (EPA)
OSHA – Occupational Safety and Health Administration
OTA – Office of Technology Assessment
OTS – Office of Toxic Substances (EPA)
PAH – polycyclic aromatic hydrocarbons
PBB – polybrominated biphenyls
PCB – polychlorinated biphenyl
PEL – permissible exposure limit
PFC – plaque forming cell
PLA – Product Licensing Application
PMN – Premanufacture Notice
POMS – Program Operations Manual System
PPB – parts per billion
PPM – parts per million
PVIZT – 1-phenyl-5-vinyl-2-imidazolidine-thione
RCRA – Resource Conservation and Recovery Act
RQ – reportable quantity
RTECS – Registry of Toxic Effects of Chemical Substances
SRBC – sheep red blood cell
SDWA – Safe Drinking Water Act
SSA – Social Security Administration
SSDI – Social Security Disability Income
SSI – Supplemental Security Income
STEL – short-term exposure limit
TCDD – 2,3,7,8-tetrachlorodibenzo-p-dioxin
TDI – toluene diisocyanate
TPQ – threshold planning quantity
TRI – Toxic Release Inventory
TSCA – Toxic Substances Control Act
TWA – time weighted average

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